DEVELOPMENT OF TRANSDERMAL DRUG DELIVERY SYSTEM FOR KETOROLAC AND OTHER ANTI-INFLAMMATORY DRUGS

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BY
AMRISH CHANDRA
M.PHARM.

INSTITUTE OF PHARMACY



BUNDELKHAND UNIVERSITY JHANSI 2009

INSTITUTE OF PHARMACY BUNDELKHAND UNIVERSITY **IHANSI**



CERTIFICATE

This is to certify that the thesis entitled "Development of Transdermal Drug Delivery System for Ketorolac and other Anti-Inflammatory Drugs" submitted to the Bundelkhand University, Jhansi (U.P.) in fulfillment of requirement for the award of Degree of Doctor of Philosophy in Pharmacy, embodies the original research work carried out by Mr. Amrish Chandra (Enrol. No. BU/2005/5675) under our supervision. This work has not been submitted in part or full for the award of any other degree of this or any other university. The candidate has put in an attendance of more than 200 days.

Prof. (Dr.) P.K.Sharma

Director

arwarded R.V. Northland Institute of Pharmacy

Dadri, Greater Noida

(Gautam Budh Nagar)

Ex-Director and Head

Institute of Pharmacy

Bundelkhand University

Jhansi, U.P.

Supervisor

Reader

Institute of Pharmacy

Bundelkhand University

Jhansi) U.P.

Institute of Pharmacy Institute of Pharmacy God Supervisor

DECLARATION

I hereby declare that the thesis entitled, "Development of Transdermal Drug Delivery System for Ketorolac and other Anti-Inflammatory Drugs" embodies the results of the original research work carried out by me in the Institute of Pharmacy, Bundelkhand University, Jhansi. under the genial guidance and supervision of Prof. (Dr.) P. K. Sharma (Supervisor) and Dr. Raghuveer Irchhiaya (Co-supervisor). The same is submitted to the Bundelkhand University, Jhansi, U.P. in fulfillment for the award of the degree Doctor of Philosophy in Pharmacy.

This work has not been submitted in part or full for the award of any degree of this or any other university.

Amrish Chandra

Date: 05/03/2009



DEDICATED TO MY PARENTS & MY FAMILY

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The hardest arithmetic to master is that which enables us to count our blessings.

~ Eric Hoffer

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CONTENTS

Chapter No.	Title P	age No.
DECLARATION		i
CERTIFICATE		ii
ACKNOWLEDGEMEN	Т	iii
CONTENTS		viii
LIST OF TABLES		xv
LIST OF FIGURE		xvii
1. INTRODUCTION	•	1
2. LITERATURE REVI	EW	6
2.1 TRANSDERMAL DR	RUG DELIVERY SYSTEM	6
2.1.1 Definition		6
2.1.2 Benefits and limitati	ons of transdermal drug delivery systems	6
2.2 DEVELOPMENT OF	TRANSDERMAL DRUG DELIVERY SYSTE	MS 8
2.2.1 Transdermal drug de	elivery system available in the market	9
2.2.2 Approaches for deve	eloping transdermal drug delivery systems	11
2.2.3 Components of typic	cal transdermal system	17
2.2.4 Gels		20
2.2.5 Microemulsion		24
2.2.6 Proniosomes		29
2.3 SKIN		33
2.3.1 Epidermis		34
2.3.2 Dermis		35
2.3.3 Hypodermis		36
2.3.4 Percutaneous absorp	otion	36
2.3.5 Skin as permeation l	barrier	39
2.3.6 Factors affecting tra	nsdermal permeation	42
2.3.7 Permeation enhance	ment	44
2.3.8 Determining skin pe	ermeation	58

I	nd	ex

2.4 THERAPEUTIC APPLICATIONS OF NSAID's	60
2.4.1 Inflammation	60
2.4.2 Pain	61
2.4.3 Rheumatoid arthritis	63
2.4.4 Osteoarthritis	64
2.4.5 Role of prostaglandins	65
2.4.6 Clinical approach	66
2.4.7 Non-steroidal anti inflammatory drugs (NSAID's)	68
3. OBJECTIVE AND PLAN OF WORK	72
3.1 AIM OF RESEARCH	72
3.2 OBJECTIVE OF STUDY	73
3.3 RATIONALE OF THE STUDY	74
3.3.1 Hydrogel based transdermal drug delivery of ketorolac	74
3.3.2 Microemulsion based gel for transdermal delivery of dexamethasone	76
3.3.3 Proniosomal based transdermal drug delivery of piroxicam	78
3.4 CHOICE OF THE DRUG	80
3.4.1 Ketorolac	81
3.4.2 Dexamethasone	82
3.4.3 Piroxicam	83
3.5 DRUG PROFILE	84
3.5.1 Ketorolac (tromethamine salt)	84
3.4.2 Dexamethasone	87
3.5.3 Piroxicam	89
3.6 EXCIPIENT PROFILE	91
4. EXPERIMENTAL - HYDROGEL BASED TRANSDERMAL DRUG DELIVERY SYSTEM OF KETOROLAC	108
4.1 MATERIALS	108
4.2 CHARACTERIZATION OF KETOROLAC	108
4.2.1 Physical characteristics of ketorolac (tromethamine salt)	108
4.2.2 Identification test	109
4.2.3 Other tests	110
4.2.4 Identification of impurities	110

4.3 ANALYTICAL METHODOLOGY	112
4.3.1 U.V. absorption spectrometric studies	112
4.3.2 Comparison of UV absorption spectra before and after storage of ketorolac PBS pH 7.4	in 113
4.3.3 Calibration curve of ketorolac (tromethamine salt)	114
4.3.4 Method for measurement of ketorolac in permeation studies	116
4.3.5 Analysis of drug content in the ketorolac gel formulation	117
4.3.6 Analysis of stability study samples	118
4.4 PREPARATION OF KETOROLAC FORMULATION	119
4.4.1 Preparation of ketorolac gel system	119
4.4.2 Fabrication of patch of ketorolac gel system	119
4.4.3 Method of application of the transdermal system	121
4.4.4 Preparation of abrasive gel	121
4.4.5 Method of application of abrasive gel	121
4.5 IN-VITRO SKIN PERMEATION STUDIES OF KETOROLAC GEL PREPARATIONS	122
4.5.1 Preparation of rat skin	122
4.5.2 Fabrication of apparatus for <i>in-vitro</i> skin permeation studies	122
4.5.3 <i>In-vitro</i> skin permeation studies of ketorolac gel TDS preparations	124
4.5.4 Effect of pH on <i>in-vitro</i> skin permeation of ketorolac through rat skin	125
4.5.5 Effect of various alcohols on <i>in-vitro</i> skin permeation of ketorolac	125
4.5.6. Effect of permeation enhancers on <i>in-vitro</i> skin permeation of ketorolac	126
4.5.7 Effect of abrasion of skin on in-vitro skin permeation of ketorolac	126
4.6 HISTOLOGICAL STUDIES	127
4.6.1 Effect of formulation on the histology of rat abdominal skin	127
4.7 PHARMACOLOGICAL STUDIES	127
4.7.1 Animal studies	127
4.7.2 Skin irritation study	128
4.7.3 Acetic acid induced writhing effect	128
4.7.4 Carrageenan induced paw edema	129
4.8 DATA ANALYSIS	130
4.8.1 Calculation for predicting desired flux (K ₀) required to be achieved for attaceffective plasma concentration	ining

4.8.2 Data analysis of in-vitro permeation studies	131
4.8.3 Statistical analysis	132
5. EXPERIMENTAL – MICROEMULSION GEL BASED TRANSDERMA DELIVERY SYSTEM OF DEXAMETHASONE	L 133
5.1 MATERIALS	133
5.2 CHARACTERIZATION OF DEXAMETHASONE	133
5.2.1 Physical characteristics of the drug	133
5.2.2 Identification test	134
5.2.3 U.V. absorption spectrometric studies	135
5.2.4 Other tests	135
5.3 ANALYTICAL METHODOLOGY	136
5.3.1 U.V. absorption spectra	136
5.3.2 Comparison of UV absorption spectra before and after storage of dexamethasone in 20% w/w PEG 400 and PBS pH 7.4 solution	137
5.3.3 Calibration curve of dexamethasone	138
5.3.4 Method for measurement of dexamethasone in in-vitro drug release studies	139
5.3.5 Analysis of dexamethasone content in the gel formulation	140
5.4 PRE-FORMULATION STUDIES FOR PREPARATION OF DEXAMETHASONE FORMULATION	141
5.4.1 Pseudoternary phase diagram study	141
5.4.2 Screening of microemulsions	142
5.5 PREPARATION OF MICROEMULSION BASED DEXAMETHASONE FORMULATION	143
5.5.1 Preparation of microemulsion of dexamethasone	143
5.5.2 Preparation of microemulsion based dexamethasone gel preparations	144
5.5.3 Fabrication of reservoir type patch of microemulsion gel of dexamethasone	144
5.5.4 Method of application of the transdermal system	146
5.5.5 Preparation and method of application of abrasive gel	146
5.6 CHARACTERIZATION OF DEXAMETHASONE LOADED MICROEMULSION PREPARATION	147
5.6.1 Droplet size determination	147
5.6.2 Viscosity measurements of microemulsion based hydrogel	147
5.6.3 Stability studies	147
5.6.4 pH measurements	148

5.7 IN-VITRO SKIN PERMEATION STUDIES OF MICROEMULSION BAS DEXAMETHASONE GEL PREPARATIONS	SED 148
5.7.1 Preparation of rat skin	148
5.7.2 Fabrication of apparatus for in-vitro skin permeation studies	149
5.7.3 In vitro skin permeation studies of dexamethasone across rat skin	150
5.8 PHARMACOLOGICAL STUDIES	152
5.8.1 Animal studies	152
5.8.2 Skin irritation study	152
5.8.3 In vivo anti-inflammatory studies	153
5.9 DATA ANALYSIS	154
5.9.1 Data analysis of <i>in-vitro</i> permeation studies	154
5.9.2 Statistical analysis	155
6. EXPERIMENTAL – PRONIOSOMAL GEL BASED TRANSDERMAI DRUG DELIVERY SYSTEM OF PIROXICAM	156
6.1 MATERIALS	156
6.2 CHARACTERIZATION OF PIROXICAM	156
6.2.1 Physical characteristics of the drug	156
6.2.2 Identification test	157
6.3 ANALYTICAL METHODOLOGY	158
6.3.1 UV absorption spectrometric studies	158
6.3.2 Comparison of UV absorption spectra before and after storage of piroxic 30% PEG 400 in PBS pH 7.4	am in 159
6.3.3 Calibration curve of piroxicam	160
6.3.4 Method for measurement of piroxicam in <i>in-vitro</i> drug release studies	162
6.3.5 Analysis of drug content in the piroxicam gel formulation	163
6.4 PREPARATION OF PIROXICAM FORMULATION	164
6.4.1 Conventional proniosome	164
6.4.2 Maltodextrin based proniosome	164
6.4.3 Sorbitol based proniosomes	165
6.4.4 Preparation of piroxicam niosomal gel	165
6.4.5 Preparation of piroxicam carbopol gel	166
6.4.6 Fabrication of patch of piroxicam gel system	166
6.4.7 Method of application of the transdermal system	168

6.5 EVALUATION	168
6.5.1 Encapsulation efficiency	168
6.5.2 Polarized microscopy and vesicle size analysis	169
6.5.3 Scanning electron microscopy (SEM)	169
6.6 IN-VITRO SKIN PERMEATION STUDIES OF PIROXICAM GEL PREPARATIONS	170
6.6.1 Preparation of rat skin	170
6.6.2 Fabrication of apparatus for in-vitro skin permeation studies	170
6.6.3 In-vitro permeation studies of piroxicam across rat skin	171
6.7 PHARMACOLOGICAL STUDIES	173
6.7.1 Animal studies	173
6.7.2 Skin irritation study	173
6.7.3 Carrageenan induced paw edema	174
6.8 DATA ANALYSIS	175
6.8.1 Calculation for predicting desired flux (K_0) required to be achieved for attaleffective plasma concentration	ining 175
6.8.2 Data analysis of in-vitro permeation studies	176
6.8.3 Statistical analysis	177
7. RESULTS AND DISCUSSION - HYDROGEL BASED TRANSDERMA SYSTEM OF KETOROLAC	L 178
7.1 IN-VITRO SKIN PERMEATION STUDIES OF KETOROLAC GEL PREPARATIONS	178
7.1.1 Effect of pH on in-vitro skin permeation of ketorolac through rat skin	178
7.1.2 Effect of various alcohols on in-vitro skin permeation of ketorolac	179
7.1.3 Effect of permeation enhancers on <i>in-vitro</i> skin permeation of ketorolac	183
7.1.4 Effect of abrasion of skin on in-vitro skin permeation of ketorolac	188
7.2 HISTOLOGICAL STUDIES	188
7.3 PHARMACOLOGICAL STUDIES	191
7.3.1 Skin irritation study	191
7.3.2 Acetic acid induced writhing effect (Antinociceptive response)	191
7.3.3 Carrageenan induced paw edema	192
8. RESULTS AND DISCUSSION – MICROEMULSION GEL BASED TRANSPERMAL DELIVERY SYSTEM OF DEXAMETHA SONE	105

8.1 PRE-FORMULATION STUDIES FOR PREPARATION OF DEXAMETHASONE FORMULATION	195
8.1.1 Pseudoternary phase diagram study	195
8.1.2 Screening of oils for microemulsions	193
	197
8.2 CHARACTERIZATION OF DEXAMETHASONE LOADED MICROEMULSION PREPARATION	198
8.2.1 Droplet size determination of microemulsion	198
8.2.2 Viscosity measurements of microemulsion based hydrogel	198
8.2.3 Conductivity measurements of microemulsion	199
8.2.4 Stability studies	199
8.2.5 pH measurements	200
8.3 IN-VITRO SKIN PERMEATION STUDIES OF MICROEMULS DEXAMETHASONE GEL PREPARATIONS	SION BASED 200
8.3.1 In-vitro skin permeation studies of dexamethasone across rat ski	in 200
8.4 PHARMACOLOGICAL STUDIES	207
8.4.1 Skin irritation study	207
8.4.2 In vivo anti-inflammatory studies	208
9. RESULTS AND DISCUSSION - PRONIOSOME BASED TRA SYSTEM OF PIROXICAM	NSDERMAL 210
9.1 EVALUATION	210
9.2 IN-VITRO SKIN PERMEATION STUDIES OF PIROXICAM OF PREPARATIONS	SEL 216
9.2.1 In-vitro permeation studies of piroxicam across rat skin	216
9.3 PHARMACOLOGICAL STUDIES	217
9.3.1 Skin irritation study	217
9.3.2 Carrageenan induced paw edema	218
10. COMPARATIVE EVALUATION	219
10.1.1 Permeation barriers	219
10.1.2 Drug stability studies	226
10.1.3 Model fitting	227
11. SUMMARY AND CONCLUSION	228
12. REFERENCES	233
13. PATENTS AND PUBLICATIONS	252

LIST OF TABLES

Table	No. Title	Page N	lo.
Table 1 -	Marketed transdermal patches		10
Table 2 -	Peaks in I.R. spectrum indicating various functional groups of sample	ketorolac	110
Table 3 -	Composition of buffer solution employed in experiments		113
Table 4 -	Absorbance values of ketorolac in phosphate buffer saline pH 2 $$ λmax 324 nm	7.4 at	115
Table 5 -	Drug content and percent recovery of ketorolac from transdern patches	nal	118
Table 6 -	Peaks in I.R. spectrum indicating various functional groups of dexamethasone sample		135
Table 7 -	Absorbance values of dexamethasone in 20% w/w PEG 400 at pH 7.4 solution at λ_{max} of 244 nm	nd PBS	138
Table 8 -	Drug content and percent recovery of dexamethasone from tra- patches	nsdermal	141
Table 9 -	Compositions of the selected microemulsion of dexamethas on w/w).	e (%	143
Table 10	- Peaks in I.R. spectrum indicating various functional groups of piroxicam sample	f	158
Table 11	- Absorbance values of piroxicam in 30% w/w PEG 400 in PB at λ_{max} of 356 nm	S pH 7.4	161
Table 12	- Drug content and percent recovery of piroxicam from transde patches	ermal	163
Table 13	- Composition of proniosomal formulation of piroxicam.		166
Table 14	- Permeation data of <i>in-vitro</i> studies of hydrogel based transde system across rat abdominal skin	rmal	186
Table 15	- Visual observation score for ketorolac transdermal formulation	ons.	191
Table 16	- Solubility of dexamethasone in microemulsions of various of $^{\circ}C$ (mean \pm S.D., n=4).	ls at 25	197
Table 17	- Physicochemical parameters of tested dexamethasone loaded microemulsions.	i	200
Table 18	- Permeation parameters of the dexamethasone loaded microer based gel transdermal system.	nulsions	204

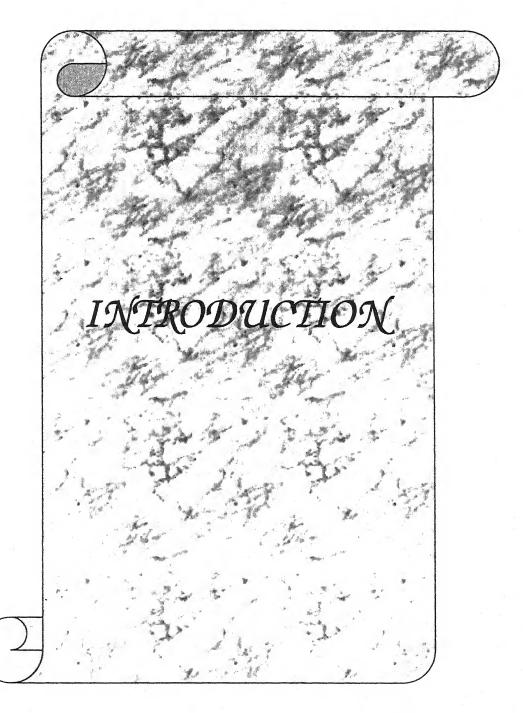
Table 19 - Enhancement ratios of various transdermal formulations of dexamethasone with respect to each other.	204
Table 20 - Visual observation score for microemulsion gel based transdermal dexamethasone formulation.	207
Table 21 - Visual observation score for piroxicam transdermal formulations.	217
Table 22 - Comparison of <i>in-vitro</i> permeation parameters for different permeation barriers	223
Table 23 - Coefficient of correlation (r)	227
Table 24 - Coefficient of determination (r ²)	227

LIST OF FIGURES

Figure No.	Title	Page No.
Figure 1 - Typic	al transdermal drug delivery system design	16
Figure 2 - Diagra	ammatic representation of the physiology of the skin	34
Figure 3 - Prosta	glandin and thromboxane synthesis pathway.	69
Figure 4 - I.R. s	pectrum of ketorolac sample	109
Figure 5 - HPLC	Cchromatograph of ketorolac	111
Figure 6 - U.V.	absorption spectra of ketorolac tromethamine in PBS pF	H 7.4 112
Figure 7 - U.V.	absorption spectra of ketorolac tromethamine portions	114
_	ration curve of ketorolac in phosphate buffer saline of ax of 324 nm	pH 7.4 at
Figure 9 - Desig	n of transdermal patch of ketorolac	120
-	esign of Keshary Chien diffusion cell used in in- meation studies	vitro skin 123
Figure 11 - I.R.	spectrum of dexamethasone sample	134
Figure 12 - U.V.	. absorbtion spectra of dexamethasone	136
Figure 13 - U.V to li	. absorbance spectra of dexamethasone before and after ght	r exposure 137
_	. absorption spectra of dexamethasone in 20% w/w PEG pH 7.4 solution at λ_{max} of 244 nm	G 400 and 139
	ign of transdermal patch of microemulsion based dexar formulation	methasone 145
	esign of Keshary Chien diffusion cell used in in- neation studies	vitro skin 149
Figure 17 - I.R.	spectrum of piroxicam sample	157
Figure 18 - U.V pH	7. absorption spectra of piroxicam in 30% w/w PEG 407.4	00 PBS of 159
Figure 19 - Abs	sorbance spectra of dexamethasone before and after ext	xposure to 160
	ibration curve of piroxicam in 30% w/w PEG 400 in F at λ_{max} of 356 nm	PBS of pH 162
Figure 21 - Desi	ign of transdermal patch of piroxicam	167
	esign of Keshary Chien diffusion cell used in in- meation studies	vitro skin 171

Figure 23 -	<i>In-vitro</i> permeation profile of ketorolac through rat abdominal skin from gel system containing ketorolac (2% w/w), HPMC (2%w/w) and formulated using PBS solutions of different pH	179
Figure 24 -	<i>In-vitro</i> permeation profile of ketorolac through rat abdominal skin from gel system containing ketorolac (2% w/w), HPMC (2%w/w) and different alcohols at 5%w/w concentration formulated using PBS solution of pH 5.4	181
Figure 25 -	In-vitro permeation profile of ketorolac across rat abdominal skin from gel system containing ketorolac (2%w/w), HPMC (2%w/w) and IPA at varying concentrations formulated in PBS solution of pH 5.4.	182
Figure 26	- Flux for ketorolac across rat abdominal skin from gel system containing ketorolac (2%w/w), HPMC (2%w/w) and IPA at varying concentrations formulated in PBS solution of pH 5.4.	182
Figure 27 -	<i>In-vitro</i> permeation profile of ketorolac through rat abdominal skin from ketorolac transdermal gel system containing ketorolac (2% w/w), HPMC (2%w/w), IPA (25%w/w) and different enhancers formulated using PBS solution of pH 5.4.	187
Figure 28 -	Enhancement ratio of ketorolac across rat abdominal skin from gel system containing ketorolac (2%w/w), HPMC (2%w/w), IPA (25%w/w) and eucalyptus oil at varying concentrations formulated in PBS solution of pH 5.4.	187
Figure 29	- Histological findings after treatment of rat abdominal skin for 24 h with chemical permeation enhancer gel system observed under microscope after staining with hematoxylin-eosin stain. A – Control, B – d-limonene. C – DMSO, D – Eucalyptus oil, E – Transcutol and F – Application of eucalyptus oil preparation after pretreatment.	
Figure 30	- Antinociceptive response of ketorolac 15 min after induction of writhes with the help of acetic acid (20mg/kg; 2ml/kg) after (i) oral administration of ketorolac and (ii) on application of transdermal patch of ketorolac (TDS) without pretreatment and (iii) after pretreatment with abrasive gel preparation.	
Figure 31 -	Anti-inflammatory activity of ketorolac on paw edema induced with carrageenan injection (0.05 ml of 0.5%w/w) in rats (Control) and after oral administration of ketorolac solution (2mg/kg) (Oral) and on application of transdermal patch of ketorolac (TDS) without pretreatment and after pretreatment with abrasive gel preparation.	
Figure 32 -	Pseudo-ternary phase diagrams of the oil, surfactant-water system at the 1:7:2 weight ratios of oil, egg lecithin-IPA mixture and distil water at 25°C (Shaded portion represents microemulsion region).	

Figure 33 - Permeation profiles of dexamethasone through excised rat skins from microemulsions based gel formulations of different oils without pretreatment.	
Figure 34 - Permeation profiles of dexamethasone through excised rat skins from microemulsions based gel formulations of different oils after pretreatment.	t
Figure 35 - Anti-inflammatory activity of microemulsion based gel of dexamethasone.	209
Figure 36 - Mean size distribution of piroxicam niosomes prepared from S2, S4, S6 and S8.	212
Figure 37 - Encapsulation efficiency of various piroxicam niosomes (%EE±S.D.).	; 214
Figure 38 - SEM images of proniosomes and polarized microscopic view of niosomes (40x): conventional (A & B), maltodextrin based (C & D), sorbitol based (E & F).	
Figure 39 - Cumulative amount of drug permeated through rat abdominal skir from different piroxicam noisomal gel.	217
Figure 40 - Anti-inflammatory studies of proniosomal gel based piroxicam transdermal system	1 218
Figure 41 - <i>In-vitro</i> permeation profile of hydrogel based transdermal system of ketorolac across different permeation barriers	f 220
Figure 42 - <i>In-vitro</i> permeation profile of microemulsion gel based transderma system of dexamethasone across different permeation barriers	1 220
Figure 43 - <i>In-vitro</i> permeation profile of proniosomal gel based transderma system of piroxicam across different permeation barriers	1 221
Figure 44 - SEM view of dorsal outer side of shed snake skin	225
Figure 45 - SEM view of dorsal inner side of shed snake skin	225
Figure 46 - Log percent drug remaining against time plot for transderma formulations	I 226



1. INTRODUCTION

In the past years, drugs have been formulated as ointments, creams or lotions for application to wounded, infected or otherwise traumatized skin surfaces to evince a "local" effect. A more esoteric mode of dosage is to deliver therapeutic agents across intact skin. In the past three decades transdermal drug delivery has moved to a phase of clinical reality beginning with the first scopolamine patches being approved in 1979, to the point where transdermal delivery system delivers a number of drugs. Presently several classes of drugs are under investigation to determine their potential for TDS (trans dermal system) development. The penetration through stratum corneum (SC) is the rate-limiting step for delivery of most of the drugs. Some of the earliest contributions related to transdermal delivery involved understanding the principal permeation barrier in the skin. As early as 1924, Rein hypothesized that the principal resistance to transdermal transport was in a layer of cells joining the stratum corneum to the epidermis.² Blank, 1964, proved the case by stripping experiments in which he removed the stratum corneum from the skin's surface and showed that the rate of water loss from skin increased dramatically once the last cellular layer of the stratum corneum was eliminated.³ Scheuplin's work⁴ established that transdermal penetration was limited by the stratum corneum itself; and this has lead to considerable towards different percutaneous activity penetration enhancement technologies.⁵ Michaels et al., 1975, did elegant experiments where they examined diffusion coefficients of different drugs through the stratum corneum showing that a number of these drugs had significant permeability.⁶ There is little question that the skin represents a very important route of delivery in that it can provide an effective means for delivering drugs that are destroyed by the liver when taken orally. The primary pathway of transdermally delivered drugs is paracellular, i.e., around the cells, then through the elastin glue. The glue-like compound, elastin, composed of collagen and hyaluronic acid and other lipids, which occupies the interstices between the cells of the top-most layer of the skin (i.e., the epidermis, including, e.g., stratum corneum, lucidum, granulosum, spinosus) must be dissolved (or otherwise disrupted) in order for a medicament or other active agent, dissolved in a solvent, to transmigrate through viable skin to the subcutaneous tissues where the cutaneous plexi of the capillary net can be reached and/or deeper penetration achieved. When the elastin is dissolved, other agents may then trans-migrate the outer layers, so the body immediately begins to attempt to repair the damage caused by the dissolution.

Diffusivity of a drug molecule is dependent on properties of both the medicament and the medium (carrier). The diffusivity in liquid media in general, tends to decrease with increased molecular volume.

The rate of skin penetration is a function of (1) the diffusion coefficient, (2)the barrier partitioning tendencies, (3) binding affinities and (4) the rate of

metabolism of the medicament by the skin. The Diffusion Coefficient of the medicament is influenced by (1) molecular weight, (2) molecular structure, (3) additives and (4) rate of metabolism of the medicament by the skin. Diffusion is also dependent on the carrier, with diffusivity decreasing with increased molecular volume.

An optimum HLB (hydrophilic lipophilic balance) is required for a medicament to penetrate efficiently. The optimum HLB may be predicted by plotting the log (permeability coefficient) vs. Log o/w (oil and water partition Coefficient) of the medicament for the stratum corneum and the viable skin. Highly lipophilic drugs bind readily in the viable skin and therefore, dissolution into the blood is minimal. Therefore, highly lipophilic drugs must be shielded to inhibit such binding. Skin metabolizes drugs effectively, so metabolism issues in the skin, such as, enzyme saturation or inhibition, medicament/metabolite fluxes (e.g., how rapidly and completely does the drug metabolize to a different form) should be taken into account. Un-ionized species of medicaments transmigrate more readily. Generally, un-ionized species are two orders of magnitude more permeable than their ionized form. Thus, the challenge of creating an effective transdermal delivery system would ultimately involve not only having a high enough drug permeability through the stratum corneum, but many other factors including ensuring that the drug delivery system does not irritate the skin, the drug was not unduly metabolized, the drug delivered in this manner bears

pharmacokinetics and pharmacodynamics and that the drug was stable in the formulation.

Today there exists a number of transdermal patches for drugs such as scopolamine, nitroglycerin, nicotine, clonidine, fentanyl, estradiol, testosterone, lidocaine, and oxybutinin.⁶ Several TDS have been successfully developed and approved by FDA for marketing, e.g., Transderm-NitroTM, EstradermTM, DuragesicTM.^{7, 8}

Transdermal clonidine, nitroglycerin and fentanyl patches exhibit fewer adverse effects than conventional oral dosage forms. Of particular note has been the value of nicotine patches in preventing smoking and prolonging life. For example, 2 years after being on transdermal nicotine patches for 12 weeks, four times as many patch wearers did not smoke compared to patients who received placebos. Of

The current U.S. market for such patches is over \$3 billion annually. Depending on the drug, the time of duration of delivery is generally from 1 to 7 days. Patches have been useful in enabling new therapies and in reducing first pass effects. For example, transdermal estradiol patches are used by over a million patients per year in contrast to oral formulations, are not associated with liver damage.

Nonetheless, only a limited number of molecules have been successfully delivered transdermally to date, so various approaches such as chemical enhancers, electricity, ultrasound and microneedles are being explored.

Iontophoresis has been the primary electrical approach studied and has been shown to provide enhanced transport for some low molecular weight molecules such as pain medications and even decapeptides. ¹¹ It is also being used as a means of extracting substances such as glucose from interstitial fluid. ¹² Electroporation, which involves higher voltage pulses for shorter time periods, has been used to temporarily create pores in the skin and has allowed the delivery of even larger molecules such as heparin and oligonucleotides through human cadaver skin. Ultrasound, particularly at low frequencies, has been shown to greatly enhance the flux of large molecular weight substances through the skin; over five thousand times normal fluxes have been achieved for molecules the size of insulin or larger and ultrasound is currently in clinical trials for delivery of insulin and pain medications. ¹³

The physicochemical properties, potency and pharmacodynamics of the drug need to be appropriate, with high potency (low dose) as a pre-requisite. With respect to other therapeutic areas, the motion-sickness drug, scopolamine is low dose, and has appropriate skin permeation characteristics to enable formulation as a transdermal patch. Peaks and troughs and their associated side effects are obviated by the slow delivery via the transdermal route. The transdermal administration of drug for systemic therapy has thus attracted much attention. The delivery of drugs through the skin for systemic effects may have several advantages over conventional oral and the other invasive methods of drug delivery.¹⁴

VIE

2. LITERATURE REVIEW

2.1 TRANSDERMAL DRUG DELIVERY SYSTEM

2.1.1 Definition

Transdermal permeation or percutaneous absorption can be defined as the passage of a drug from the outside of the skin through its various layers into the bloodstream and this term includes all topically administered drug formulations.

2.1.2 Benefits and limitations of transdermal drug delivery systems

Certainly, each dosage form has its unique place in medicine but some attributes of the transdermal drug delivery system provide distinct advantages over the traditional methods of attaining systemic levels of drugs.^{15,16}

Advantages

- The system avoids chemically hostile gastrointestinal environment.
- Doesn't have gastrointestinal distress or other physiologic contraindications of oral route.
- Provides controlled administration of a therapeutically effective dose at a desired rate of delivery.
- Allows effective use of drugs with short biological half-lives.
- Allows administration of drugs with narrow therapeutic window.

- Maintains drug concentration within an optimal therapeutic range even during prolonged therapy.
- Leads to the reduction of adverse drug effects.
- Maximizes the efficacy dose relationship.
- Minimizes the need for frequent drug administration.
- Leads to better patient compliance due to reduction in the frequency of dosing.
- Can be administered to patients who frequently miss doses.
- By passes hepatic first pass metabolism.
- Interrupts drug input promptly when toxicities occur (Termination of drug therapy by removal of patch).
- Possesses better cost: benefit ratio.
- Self-application is possible.

Limitations

- Drugs that require high-blood level can not be administered.
- Adhesive may not adhere well to all types of skin.
- Drug or drug formulation may cause skin irritation or sensitization.
- System may not be economical.
- Difficulty of permeation through human skin for some drugs, e.g., high molecular weight drugs.
- Cutaneous metabolism may occur.

2.2 DEVELOPMENT OF TRANSDERMAL DRUG DELIVERY SYSTEMS

Several technical approaches have been used to provide rate control over the release and transdermal permeation of drugs. All such transdermal dosage forms typically have a basic structure comprising many layers, with each having a specific function.

- An outer covering (barrier): This backing layer is the outer most layer of the transdermal system which prevents wetting of the system.
- A drug reservoir: The second layer contains a reservoir that supplies a continuous quantum of drug for the predetermined functional lifetime of the system.
- Rate control polymeric membrane: It regulates the rate of drug delivery from the drug reservoir to the skin surface. However, some transdermal systems lack a rate control membrane and the drug diffuses directly from the reservoir to the skin surface.
- Adhesive layer: Which is applied to part or whole of the system/skin interface and it contains a small fraction of the drug to deliver the initial loading dose and glues the system to the skin. The adhesive layer is covered with a protective release liner that protects the integrity of the dosage form and is removed before the use of the transdermal system.

The activity of these systems is defined in terms of the rate of release of drug from the system.

Transdermal drug delivery system provides rate controlled continuous supply of the drug during a predetermined time interval. The drug so delivered diffuses through the skin and enters the systemic circulation, bypassing the liver and the rate of drug release from the transdermal device is normally a small fraction of the amount that the skin can possibly absorb. Hence, even if there are variations in skin permeability, a constant rate of drug input into the circulation is achieved.

2.2.1 Transdermal drug delivery system available in the market

The commercialization of transdermal patches for controlled drug delivery began three decades ago and has resulted in diverse products. Among them are nitroglycerine for angina, 17 scopolamine for prophylaxis or therapy of motion sickness, 18 nicotine for smoking cessation, 19 estradiol for hormone replacement therapy. 20 testosterone for male hypogonadism, 21 clonidine for hypertension. 22 Examples of marketed transdermal patches are mentioned in Table 1.

Table 1 - Marketed transdermal patches

Brand Name (Active Drug)	Patch type	Duration of Application
Alora (Estradiol)	Matrix	3 to 4 days
Androderm (Testosterone)	Membrane	24 hours
CatapresTTS (Clonidine)	Membrane	7 days
Climara (Estradiol)	Matrix	7 days
CombiPatch (Estradiol/Norethindrone acetate)	Matrix	3 to 4 days
Duragesic (Fentanyl)	Membrane	72 hours
Esclim (Estradiol)	Matrix	3 to 4 days
Estraderm (Estradiol)	Membrane	3 to 4 days
Minitran (Nitroglycerin)	Matrix	12 to 16 hours
Nicoderm CQ (Nicotine)	Membrane	24 hours
Nicotrol (Nicotine)	Matrix	16 hours
Nitradisc (Nitroglycerin)	Matrix	24 hours
Nitro-Dur (Nitroglycerin)	Matrix	12 to 16 hours
Nitroglycerin Generic (Nitroglycerin)	Matrix	12 to 16 hours
Ortho-Evra (Norelgestromin/Ethynil estradiol)	Matrix	7 days
Testoderm (Testosterone)	Membrane	24 hours
Transderm-Nitro (Nitroglycerin)	Membrane	12 to 16 hours
Transderm-Scop (Scopolamine)	Membrane	72 hours
Vivelle (Estradiol)	Matrix	3 to 4 days
Vivelle-Dot (Estradiol)	Matrix	3 to 4 days

2.2.2 Approaches for developing transdermal drug delivery systems

Several technologies have been successfully developed to provide rate control over the release and skin permeation of drugs. Four different approaches have been utilized to manufacture transdermal patches.^{23,24}

Polymer membrane permeation-controlled systems

In this system the drug reservoir is sandwiched between a drug impermeable metallic plastic laminate and a rate-controlling polymeric membrane, which may be micro porous or non-porous, e.g., ethylene-vinyl acetate (EVA) copolymer, with a defined drug permeability property (Fig. 1A). The drug molecules are permitted to release only through the rate-controlling polymeric membrane. In the drug reservoir compartment, the drug solids are dispersed homogeneously in a solid polymer matrix (e.g., poly isobutylene), suspended in an unreachable, viscous liquid medium (e.g., silicone fluid) to form a paste like suspension or dissolved in a releasable solvent (e.g., alkyl alcohol) to form a clear drug solution. On the external surface of the polymeric membrane a thin layer of drug compatible, hypoallergenic pressure sensitive adhesive polymer, e.g., silicone adhesive, may be applied to provide intimate contact of the system with the skin surface. The rate of drug release from this system can be tailored by varying the composition of the drug reservoir formulation and the permeability coefficient and/or thickness of the rate controlling membrane and the adhesive.

The intrinsic rate of drug release (dQ/dt) from this system should be constant and defined by the following equation:

$$\frac{dQ}{dt} = \frac{K_{m/r} K_{a/m} D_a D_m}{K_{m/r} D_m h_a + K_{a/m} D_a h_m} C_R$$

Where C_R is the drug concentration in the reservoir compartment; $K_{m/r}$ and $K_{a/m}$ partition coefficients for the interfacial partitioning of drug from the reservoir to the membrane and from the membrane to the adhesive layer, respectively; D_m and D_a are diffusion coefficients in the rate controlling membrane with thickness of h_m and in the adhesive layer with a thickness of h_a respectively. For a micro porous membrane the porosity and tortuosity of the membrane should also be taken into account in calculation of the D_m and h_m values.

The constant release rate of the drug is the major advantage of membrane permeation controlled transdermal system. However, a rare risk also exists when an accidental breakage of the rate controlling membrane can result in dose dumping or a rapid release of the entire drug content.

PolymermMatrix dispersion controlled transdermal delivery system

In this system, the drug reservoir is prepared by homogeneously dispersing drug particles in a hydrophilic or lipophilic polymer matrix (Liquid polymer or a highly viscous base polymer). The resultant medicated polymer is then molded into a medicated disc with a defined surface area and controlled thickness.

This drug reservoir-containing polymer disc is then pasted on to an occlusive base plate in a compartment fabricated from a drug impermeable plastic backing. The adhesive polymer is then spread along the circumference to form a strip of adhesive rim around the medicated disc. (Fig. 1B).

The rate of drug release in this system is defined by:

$$\frac{dQ}{dt} = \left(\frac{L_d C_p D_p}{2t}\right)^{1/2}$$

Where L_d is the initial drug loading dose dispersed in the polymer matrix, C_p and D_p are solubility and diffusivity of the drug in the polymer respectively.

The advantage of the matrix diffusion is the absence of dose dumping, since the polymer cannot rupture.

Adhesive diffusion controlled system

This is a simplified form of the membrane permeation controlled system. As represented in Fig 1C, the drug reservoir is formulated by directly dispersing the drug in an adhesive polymer, made up of either poly (isobutylene) or poly (acrylate) and then spreading the medicated adhesive by solvent casting onto a flat sheet of drug-impermeable metallic plastic backing to form a thin drug reservoir layer. The layers of non-medicated rate-controlling adhesive polymer of constant thickness are applied on the top of the drug reservoir layer to produce an adhesive diffusion controlled delivery system. The rate of drug release in this system is defined by:

$$\frac{dQ}{dt} = \frac{k_{a/r} D_a C_r}{h_a}$$

Where, $K_{a/r}$ is partition coefficient for the interfacial partitioning of drug from the reservoir layer to the adhesive layer.

Microreservior dissolution-controlled transdermal drug delivery systems

This type can be considered a hybrid of the reservoir and matrix dispersion-type drug delivery systems. Here, the drug reservoir is formed by first suspending the solids in an aqueous solution of a water-miscible drug solubilizer e.g., polyethylene glycol and then homogeneously dispersing the drug suspension in a lipophilic polymer e.g. silicone elastomers, by high energy dispersing technique to form thousands of unleachable microscopic spheres of drug reservoirs (Fig. 1D). This thermodynamically unstable dispersion is quickly stabilized by immediately cross linking the polymer chains in situ, which produces medicated polymer disk with a constant surface area and a fixed thickness. A transdermal therapeutic system is then produced by mounting the medicated disk at the centre of an adhesive pad. The rate of drug release from a micro reservoir drug delivery system is defined by:

$$\frac{dQ}{dt} = \frac{D_p D_s A K_p}{D_p h_d + D_s h_p A K_p} \left[B S_p - \frac{D_1 S_1 (1 - B)}{h_1} \left(\frac{1}{K_1} + \frac{1}{K_m} \right) \right]$$

Where A= a/b, a is the ratio of the drug concentration in the bulk of elution solution over the drug solubility in the same medium; b = ratio of the drug concentration at the outer edge of the polymer coating membrane over the

drug solubility in the same polymer composition; B= ratio of the drug concentration at the inner edge of the interfacial barrier over the drug solubility in the polymer matrix, K_1 , K_m and K_p are the partition coefficients for the interfacial portioning of drug from the liquid compartment to the polymer matrix, from the polymer matrix to the polymer coating membrane and from the polymer coating membrane to the elution solution (or skin) respectively; D_1 , D_p and D_s are drug diffusivities in the liquid compartment, polymer coating membrane and elution solution (or skin) respectively. S_1 and S_p are the solubility of the drug in the liquid compartment and in the polymer matrix respectively. S_1 , S_2 , and S_3 are the thickness of the liquid layer

polymer matrix respectively. h_1 , h_p , and h_d are the thickness of the liquid layer surrounding the drug particles, the polymer coating membrane around the polymer matrix and the hydrodynamic diffusion layer surrounding the polymer coating membrane respectively. D_a is the diffusion coefficient in adhesive layer (a), C_R is the drug concentration in the reservoir compartment (R).

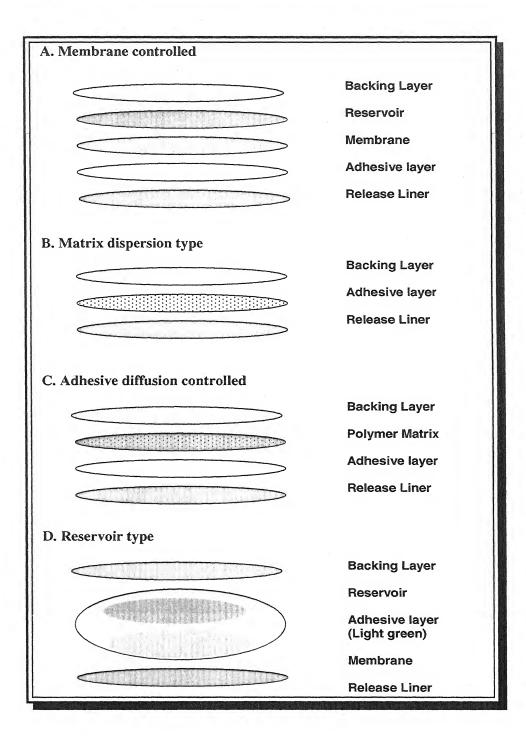


Figure 1 - Typical transdermal drug delivery system design

2.2.3 Components of typical transdermal system

Polymer

The use of polymers for skin preparations is manifold. Requirements of such polymers are dependent on the formulation type. The most applied polymers on skin belong to various classes for e.g. cellulose derivatives, chitosan, carrageenan, polyacrylates, polyvinyl alcohols, polyvinyl pyrrolidones and silicones. They are gelating agents, matrices in patches and wound dressings, antinucleants and penetration enhancers.

The polymer controls the release of the drug from the device. It should have the following desirable properties:¹⁶

- The molecular weight, glass transition temperature and chemical functionality should be suitable so that the drug diffuses properly and released through it.
- 2. The polymer should be stable non reactive with the drug, easily manufactured/fabricated into the desired product and be inexpensive.
- The polymer and its degradation product should be non-toxic and nonantagonistic to the host.
- 4. The mechanical properties of the polymer should not deteriorate excessively when large amount of active agents are incorporated into it.

The polymer used may be of either type i.e. natural type or synthetic type.

Adhesives

The adhesive system should fulfill the following criteria: 16

- Should not irritate or sensitize the skin or cause an imbalance in the normal skin flora during its contact time with skin.
- Should adhere to the skin aggressively.
- Should be easily removed.
- Should not leave an un-washable residue.
- Should have an excellent contact with skin both at microscopic and macroscopic level.
- It should be compatible to the drug, excipients and enhancers of the device of which it is a part.
- Permeation of the drug should not be affected.

Solvents

Various types of solvents are used in the transdermal drug delivery system.

- Aqueous solvents: It is used because hydration has been shown to decrease the barrier function of the SC. Water has been used as a solvent in many clinically effective preparations such as ointments and patches. In general increased tissue hydration appears to increase the transdermal delivery of both hydrophilic and lipophilic permeants.
- Non-aqueous solvents: The various types of non-aqueous solvents include short chain alcohol (ethanol, propanol, isopropyl alcohol,

butanol, glycerol etc.), long chain fatty alcohols, poly alcohols (propylene glycols) etc.

Rate controlling membranes

Rate controlling membranes in transdermal penetration devices govern drug release from the dosage form. The various types of rate controlling membranes include ethylene vinyl acetate copolymers membranes, membranes made from the natural polymeric materials such as chitosan, polydroxyethylmethacrylate membranes etc. The choice of these controlling membranes is based on the requirements of the formulation.

Backing laminates

The primary function of the backing membrane is to provide support. Typical membranes are composed of pigmented layers, an aluminum vapor coated layer, a plastic film (polyethylene, polyvinyl chloride or polyester) and a heat-sealed layer. The common materials are the polyesters (Mylar®)-polyethylene co-extruded films, polypropylene and ultra low and linear low density polyethylene resins.

Release liners

It prevents the loss of the drug during storage. The release liner has to be removed before the application of the transdermal system. It also helps to prevent contamination. The materials used as release liners include paper, fabrics, polyethylene, PVC, polyesters, foil, Mylar® and other metalised laminates.

pH adjusters

These are the agents used to adjust the pH of the transdermal drug delivery system. The commonly used pH adjuster includes monoethanolamine, diethanolamine, triethanolamine, lactic acids, sodium hydroxide, sodium phosphate etc.

Description of the formulations

2.2.4 Gels

U.S.P. defines gels as semisolids, either suspensions of small inorganic particles or large organic molecules interpenetrated with liquid. ²⁵

Gels can be also defined as

- A gel is a jelly like colloidal system in which a liquid is dispersed in a solid medium. The process of gel formation is known as gelation.
- 2) Gels rich in liquid are called jellies. Jellies are transparent or translucent, non-greasy, semisolid preparation meant for external preparation to the skin or mucous membrane
- Gels are formed by aggregation of colloidal sol particles; the solid or semisolid system so formed being interpenetrated by liquid. The particle link together to form an interlaced network, thus imparting rigidity to the structure, the continuous phase is held in the meshes.

Among all the gel types, hydrogels have gained the most popularity.

Hydrogels are cross linked, three-dimensional, macromolecular polymer networks that are insoluble, but are able to swell rapidly in water or biological fluids. In the process, they can retain large volumes of water (sometimes up to 90%) in their swollen three-dimensional networks. The swollen aqueous networks are called as hydrogels.

Hydrogels are usually made of hydrophilic polymer molecules, cross linked either by chemical bonds or due to other cohesion forces such as ionic interaction, hydrophilic interaction or by hydrogen bonding. They are elastic in nature. Hydrogels can be formed by cross linking one or several types of monomer units into a network, forming a homopolymer, copolymer or multipolymer. With the incorporation of different monomers, gels with wideranging chemical and physical properties can be formed. The gels can be neutral or charged, soft or stiff, strong or brittle.

Hydrogels can be made to respond to the environment and the extent of the response can be controlled. The environmental conditions to which a hydrogel can be made responsive can be pH, temperature, electric field, ionic strength, salt type, solvent, external stress, light or a combination of these. It is because of these unique properties that these classes of polymer based systems embrace numerous pharmaceutical and biomedical applications.

Advantage

- Hydrogels exhibit good biocompatibility, low degradation and ease of processing. Hydrogels have low interfacial tension with surrounding body fluids and tissues that minimizes the driving force for protein and adsorption and cell adhesion.
- Hydrogels simulate some hydrodynamic properties of natural biological gels, cells and tissues in many ways.
- The soft rubbery nature of hydrogels minimizes frictional and mechanical irritation to the surrounding tissue.
- Release of the drug from a hydrogel is dependent on its composition, swelling capacity and cross linked density and the release can thus be regulated by controlling water swelling and cross linking density.
- Wide range of drugs (both hydrophilic as well as hydrophobic drugs, charged and neutral drugs) can be incorporated into hydrogel and the desired release can be obtained.

Limitation

The only major problem of hydrogel is their low mechanical strength and poor toughness after swelling. To reinforce their structure, hydrogel are cross linked or blended with other polymers; both the methods are convenient and effective to improve the physical and mechanical properties of hydrogel for practical use.

Classification of hydrogel

Based on method of preparation, hydrogels can be broadly classified into

- Homopolymer hydrogels: These hydrogels are made with only one type of hydrophilic monomer.
- Copolymer hydrogels: These hydrogels are made with two types of monomers, out of which at least one is hydrophilic.
- Multipolymer hydrogels: These hydrogels are made with more than three types of monomers interpenetrating polymeric hydrogels; these hydrogels are made by swelling a network of a polymer in another monomer, forming intermeshing network of the two polymers.

Based on the structure, hydrogels can be classified into

- Amorphous hydrogels (chains randomly arranged)
- Semicrystalline hydrogels (dense regions of ordered macromolecules,
 i.e. crystallites).
- Hydrogen-bonded hydrogels

Based on mechanism controlling the drug release from hydrogels, these can be classified into

- Diffusion controlled release systems.
- Swelling controlled release systems
- Chemically controlled release systems.
- Environment responsive systems.

2.2.5 Microemulsion

The term "microemulsion" refers to a thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules. A microemulsion is considered to be a thermodynamically or kinetically stable liquid dispersion of an oil phase and a water phase, in combination with a surfactant. The dispersed phase typically comprises small particles or droplets, with a size range of 5 nm-200 nm, and has very low oil/water interfacial tension. Because the droplet size is less than 25% of the wavelength of visible light, microemulsions are transparent. The microemulsion is formed readily and sometimes spontaneously, generally without high-energy input. In many cases a cosurfactant or cosolvent is used in addition to the surfactant, the oil phase and the water phase.

Types of microemulsion

Three types of microemulsions are most likely to be formed depending on the composition:

- Oil in water (o/w) microemulsions wherein oil droplets are dispersed in the continuous aqueous phase
- Water in oil (w/o) microemulsions wherein water droplets are dispersed in the continuous oil phase;

 Bi-continuous microemulsions wherein microdomains of oil and water are interdispersed within the system.

In all three types of microemulsions, the interface is stabilized by an appropriate combination of surfactants and/or co-surfactants.

How is it different from emulsion?

The key difference between emulsions and microemulsions are that the former, whilst they may exhibit excellent kinetic stability, are fundamentally thermodynamically unstable and will eventually phase separate. Another important difference concerns their appearance; emulsions are cloudy while microemulsions are clear or translucent. In addition, there are distinct differences in their method of preparation, since emulsions require a large input of energy while microemulsions do not. The latter point has obvious implications when considering the relative cost of commercial production of the two types of system.

Microemulsion formation and stability can be explained on the basis of a simplified thermodynamic rationalization. The free energy of microemulsion formation can be considered to depend on the extent to which surfactant lowers the surface tension of the oil—water interface and the change in entropy of the system such that,

$$\Delta G_{\rm f} = \gamma \Delta A - T \Delta S$$

where ΔG_f is the free energy of formation, γ is the surface tension of the oilwater interface, ΔA is the change in interfacial area on microemulsification, ΔS is the change in entropy of the system which is effectively the dispersion entropy and T is the temperature. It should be noted that when a microemulsion is formed the change in ΔA is very large due to the large number of very small droplets formed. It must however be recognized that while the value of γ is positive at all times, it is very small (of the order of fractions of mN/m) and is offset by the entropic component. The dominant favorable entropic contribution is the very large dispersion entropy arising from the mixing of one phase in the other in the form of large numbers of small droplets. However, favorable entropic contributions also arise from other dynamic processes such as surfactant diffusion in the interfacial layer and monomer-micelle surfactant exchange. Thus a negative free energy of formation is achieved when large reductions in surface tension are accompanied by significant favorable entropic change. In such cases, microemulsification is spontaneous and the resulting dispersion is thermodynamically stable.

Emulsion formers

Though it has been know that several factors determine whether a w/o or o/w system will be formed but in general it could be summarized that the most

likely microemulsion would be that in which the phase with the smaller volume fraction forms the droplets i.e. internal phase.

The surfactants used to stabilize such systems may be:

- Non-ionic
- Zwitterionic
- Cationic
- Anionic surfactants

Various pharmaceutically acceptable excipients available that can be used in microemulsion formulation are:

Long chain or high molecular weight (>1000) surfactants include:

Gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, microcrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinyl pyrrolidene (PVP).

The low molecular weight (<1000) surfactants include:

Stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, and sorbitan esters.

In microemulsions, one can design the interface of such nanometer sized droplets so that droplet stability and lifespan in humans can be made to last from a few milliseconds to minutes or even to hours. The interfacial rigidity of the microemulsion droplets plays a key role in the flux of the drugs from such droplets to the cells and tissues. Tailoring of microemulsion systems to control the flux of the drugs can be done so as to customize drug delivery according to individual patient requirements or to specific pharmaceutical needs.

It can be seen that there is a real and continuing need for the development of new and effective drug delivery systems for water insoluble or sparingly soluble drugs. One such approach might be pharmaceutical microemulsions. However, materials must be chosen that are biocompatible, non-toxic, clinically acceptable and use emulsifiers in an appropriate concentration range and form stable microemulsions. Thus the formulation developed must be safe and effective pharmaceutical microemulsion delivery systems.

Advantages of microemulsion over other dosage forms

The following are some of the prominent benefits of microemulsions:

- Increase the rate of absorption
- Eliminates variability in absorption
- Helps solublize lipophilic drug

- Provides a aqueous dosage form for water insoluble drugs
- Increases bioavailability
- Various routes like tropical, oral and intravenous can be used to deliver the product
- Rapid and efficient penetration of the drug moiety
- Provides protection from hydrolysis and oxidation as drug in oil phase
 in O/W microemulsion is not exposed to attack by water and air.
- Liquid dosage form increases patient compliance.
- Less amount of energy requirement.

2.2.6 Proniosomes

Transdermal delivery can be achieved using various colloidal carries like:

- Niosomes
- Liposomes
- Virosomes
- Proliposomes
- Proniosomes
- Transfersomes

Drug can be given in the form of gels, transdermal patches and proniosomal gels by transdermal route. Gels needs penetration enhancers for better permeation of drug through stratum corneum and these penetration enhancers may cause irritation to the skin. It is difficult to control drug release from

transdermal patches and gels, so colloidal carriers are better choice as niosomes in dispersion can act as reservoirs containing drugs and modification of the particles composition or surface can adjust the drug release rate and/or the affinity for the target site. Their advantages are outlined as:

- Niosomes produce a controlled drug release profile at a desirable rate as bilayers act as barrier.
- Drug entrapment is very high.
- Vesicles size can be controlled using surfactant according to their HLB values.
- Vesicles in contact with stratum corneum aggregate fuse and adhere to the cell surface, this interaction lead activity gradient of the drug at the vesicle stratum corneum interface is the driving force for penetration of the lipophilic drugs across the stratum corneum, thus acting as self penetration enhancers.
- Direct transfer of drug from vesicle to skin occurs when drug is intercalated within bilayers of niosomes.

Proniosomes are non-ionic based vesicular system overcomes the problems of physical instability of aqueous niosomal dispersions (aggregation, fusion and leakage), the encapsulation efficiency of proniosomes is high and they are capable of incorporating lipophilic, hydrophilic and amphiphilic drugs.²⁶

Proniosomes are categorized into major division.

- Dry granular type of proniosomes
- Liquid crystalline proniosomes (gel form)

Preparation methods are based on the fact that mixture of phospholipids, nonionic surfactants ethanol and water can be used to form a concentrated proniosomal gel which can be converted to a stable noisome suspension by the hydration with excess of aqueous phase. There are two methods namely: Slurry method²⁷ and phase coacervation method.²⁸

Drug can be added at two stages

- To the surfactant solution prior to spraying of solution on carrier (maltodextrin or sorbitol) if the drug is lipophilic.
- To the aqueous phase which is used to dissolve or hydrate the proniosomes if the drug is hydrophilic

Slurry method

Maltodextrin powder is added into round bottom flask containing surfactant solution; additionally chloroform with low concentration of surfactant is added to form slurry. Evaporation is done and vacuum is applied till dry powder is formed.

This method has maltodextrin as a carrier and process is independent of ratio of surfactant to carrier in terms of entrapment efficiency. Sorbitol is a carrier which is further coated with non-ionic surfactant. On addition of hot water and agitation it given niosomes and it is the most widely used method. It is

prepared by spraying surfactant mixture (dissolved in organic solvent) on the sorbitol powder. Evaporation of solvent is done until dry powder is formed. Since sorbitol carrier is very thin and hydration of this coating allows only slow and controlled surfactant application to avoid slurry formation too wet resulting in degradation.

Advantages

- Industrially scalable.
- Deriving niosomes from proniosomes for drug delivery is easy.
- Has large surface area resulting in thinner surfactant coating, leading to easy rehydration.
- Maltodextrin concentration can be reduced (in cases where maltodextrin reduce/affect encapsulation efficiency) to get greater surfactant and drug loading.
- Useful for hydrophobic and amphiphilic drugs.

Phase coacervation method

In this method basically, surfactant alcohol and drug are taken in a clean dry and wide mouth small glass tube. After mixing all the ingredients the open end of the glass tube is covered with a lid to prevent the loss of solvent from it, till all ingredients are dissolved. The aqueous phase is added and warmed on a water bath (60-70°C) till clear solution is obtained which on cooling, gets converted into gel, which acts as a reservoir for transdermal drug delivery.

Advantages

- Size distribution is uniform.
- Suitable method for formulating hydrophobic drugs in a lipid suspension.
- Useful when active ingredient is susceptible to hydrolysis.

2.3 SKIN

Skin is the largest organ of the body covering an area of about 2m² and accounting for more than 10% of body mass, in an average human adult. It receives approximately about one third of total blood circulating through the body.²⁹ The skin separates the underlying blood circulation network from the outside environment and serves as a barrier against physical, chemical and microbial attacks, acts as a thermostat in maintaining body temperature plays a role in the regulation of blood pressure and protects against the penetration of ultraviolet rays.

Anatomically skin (Fig. 2) can be divided into three layers:

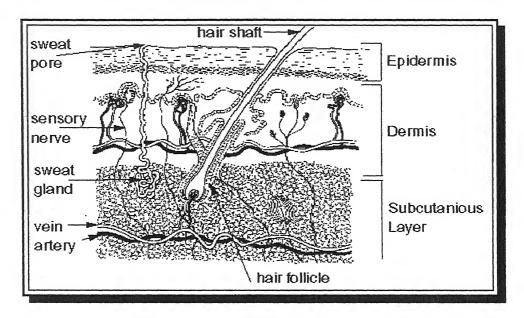


Figure 2 - Diagrammatic representation of the physiology of the skin

- The epidermis
- The dermis, which is richly supplied by lymphatic and blood vessels which and provides an efficient means of drug removal into the systemic pool.
- The hypodermis (or subcutaneous fat)

2.3.1 Epidermis

The epidermis is the outermost layer of the skin and is approximately 150 μ m thick³⁰ consisting of many layers of compact, flattened, dehydrated and keratinized cells, which act as a physical protective barrier.

The SC is the heterogeneous outermost layer of the epidermis and is approximately 1-20 µm thick. It is non-viable epidermis and consists, in a

given cross section 15-25 flattened, stacked, hexagonal and cornified cells embedded in intercellular lipid. Each cell is approximately 40 µm in diameter and 0.5 µm thick. The SC barrier properties may be related to its very high density (1.4 gm/cm³ in the dry state), its low hydration of 15-20%, compared with the usual 70% for the body and its low surface area for solute transport. The intercellular region consists mainly of lipids and desmosomes and serves to cement the structure together into a coherent membrane^{7, 31} The SC is followed by stratum lucidum, stratum granulosum, stratum spinosum and stratum germinatum.

2.3.2 Dermis

The dermis is about 3 – 5 mm thick with 0.3 mm thick being on eyelids and consists of collagenous fibers (70%) providing a scaffold of support and cushioning and elastic connective tissue providing elasticity in a semigel matrix of mucopolysaccharides. The main cells of dermis include, fibroblats, which produce the connective tissue components of collagen, laminin, fibronectin and vitronectin; mast cells, which are involved in the immune and inflammatory responses; and melanocytes involved in the production of the pigment melanin.

The dermis not only provides the nutritive, immune and other support system for the epidermis, through a thin papillary layer adjacent to the dermis but also plays a role in temperature, pressure and pain regulation. A rich bed of capillaries is encountered at 20µm in the dermal layer. It also contains the lymphatics, nerves and the epidermal appendages such as hair follicles sebaceous glands and sweat glands. Each hair follicle is associated with one or more sebaceous glands which are outgrowths of epithelial cells. The duct of the sebaceous gland is filled with a soft slowly extruded lipoidal medium, the sebum. The sweat glands are divided into the eccrine and appocrine types. Eccrine glands are particularly concentrated in the palms and soles. The appocrine types are found in the armpits, anogenital regions and nipples.

2.3.3 Hypodermis

The hypodermis is the deepest layer of the skin.³² It acts as a heat insulator, a shock absorber and an energy storage region. This layer contains a network of fat cells (possibly 50% of the body's fat), fibroblasts and macrophages. The major role of the hypodermis is to carry the vascular and neural system for skin. It also anchors the skin to underlying muscles.³³

2.3.4 Percutaneous absorption

The absorption of solutes from the skin is also called as percutaneous absorption. Percutaneous absorption involves passive diffusion of substances through the skin. The mechanism of permeation involves passage through the epidermis itself (transepidermal absorption) or through shunts (transfollicular absorption).³⁴

Basically the pathways are postulated for the absorption of the solute from the epidermis i.e. transcellular, intercellular pathways and transfollicular pathways.

Transcellular

Transport by the transcellular route involves the repeated partitioning of the molecule between the lipophilic and the hydrophilic compartments including the almost impenetrable corneccytes, intercellular matrix of keratin and keratohyaline. The polar solutes are thought to diffuse through a high energy pathway involving immobilized water near the outer surface of keratin filaments. In contrast the lipid soluble solutes tend to diffuse though a nonpolar (interstitial) lipid pathway.³⁵

Intercellular pathway

Alery and Hadgraft in 1979, suggested that tortuous intracellular diffusional pathway around keratinocytes was the preferred route of penetration through the SC rather than the drug diffusing through the keratinized cells.³⁶ Most evidence for the existence of the intercellular lipid transport pathway comes from the organization and the structure of the lipid bilayer by the observed histological localization of applied substances within these bilayer following topical application and the effect of delipidisation of these bilayer by appropriate solvents.

Histochemical studies have shown that the intracellular spaces of the SC are devoid of lipid.³⁷ Experimental evidence using precipitation of percutaneously applied compounds has led to visualization of permeation through the intracellular pathways.³⁸

The passage through the dermal region represents final hurdle to systemic entry. Permeation through the dermis is through the interlocking channels of the ground substance. Diffusion through the dermis is quiet unhindered, easy and without molecular selectivity, since gaps between the collagen fibers are far too wide to filter large molecules.³⁰

Transfollicular transport

Penetration through hair follicles involves the hair fiber itself, through the outer root sheath of the hair into the viable cells of the follicle or through the air filled canal and into the sebaceous gland. The release of sebum by the sebaceous gland may provide lipoidal pathway that may influence absorption by this route. The route for the sweat duct may involve diffusion through either the lumen or walls to below the epidermis and through the ring of keratinized cells. Dense capillary networks closely envelopes the bases of both the hair follicles and the sweat ducts providing access to the circulation for most molecules reaching these regions. Hubber et al., 1992 used the observation that a higher reservoir and permeability barrier function in

appendage-free (scar) than in normal SC as supporting evidence for a significant contribution of the appendageal route to overall skin transport. ³⁹ Recently a growing number of investigators are inclined to accept both routes, with the relative importance depending upon the characteristics of the penetrating molecules. In the initial transient diffusion stage, the drug molecules may penetrate the skin along the hair follicles or sweat ducts and are then absorbed through the follicular epithelium and the sebaceous glands. When a steady diffusion has been reached, diffusion through the stratum corneum becomes the dominant pathway.

2.3.5 Skin as permeation barrier

Before a topically applied drug can act either locally or systemically, it must penetrate the stratum corneum. This horny layer of the skin presents very peculiar physcio-chemical properties, acting both as a barrier and a reservoir. It has been said that nothing penetrates freely through this layer but everything penetrates to a certain degree.^{7, 27} Beneath the stratum corneum, the living viable epidermis is highly hydrophilic in nature and the diffusional properties of this region are somewhat similar to those of an aqueous protein gel.

The phenomenon of percutaneous absorption (or skin permeation) of a drug molecule presented in a dermal delivery system can be visualized as consisting of a series of steps in sequence.

- Diffusion of the drug molecule within the device.
- Diffusion of a penetrant molecule onto the surface layers of SC.
- Partitioning through it into the viable epidermis.
- Diffusion through the viable epidermis.
- Finally, at the papillary layer of the dermis, the molecule is taken up into the microcirculation for subsequent systemic distribution.

The viable tissue layers and the capillaries are relatively permeable and the peripheral circulation is sufficiently rapid, so that for the great majority of penetrants, diffusion through the stratum corneum is often the rate limitation step. Hence the stratum corneum acts as a passive, but not an inert, diffusion medium. No active transport process has been shown to be involved in skin permeation, therefore the mechanism of permeation follows the diffusion principles described by Fick.

There are significant differences in the structure and chemistry of human SC from one region of the body to another are reflected in the skin's permeability, For instance, plantar and palmer callus can be as thick as $400-600~\mu m$ compared to only $10-20~\mu m$ for the back, arms, legs and abdomen.

For a systemically active drug to reach a target tissue from the site of drug administration on the skin surface, it must possess physico-chemical properties that facilitate the sorption of drug by the stratum corneum, the penetration of drug through the viable epidermis and also the uptake of drug

by microcirculation in the dermal papillary layer. The rate of permeation 'dQ/dt' across various layers of skin tissue can be expressed mathematically as:

$$\frac{dQ}{dt} = P_s \left(C_d - C_r \right)$$

Where C_d and C_r are, respectively, the concentrations of a skin penetrant in the donor phase, e.g., the concentration of drug on the stratum corneum surface as delivered from transdermal drug delivery system, and in the receptor phase, e.g., systemic circulation; and P_s is the overall permeability coefficient of the skin tissues to the penetrant and is defined by:

$$P_{s} = \frac{K_{s/d} * D_{ss}}{h_{s}}$$

Where $K_{s/d}$ is the partition coefficient for the interfacial partitioning of the penetrant molecule from a transdermal drug delivery system onto the stratum corneum; D_{ss} is apparent diffusivity for the steady-state diffusion of the penetrant molecule through the skin tissues; and h_s is the overall thickness of the skin tissues for penetration.

In order to achieve a constant rate of drug permeation the drug concentration on the surface of the stratum corneum (C_d) should be consistently and substantially greater than the drug concentration in the body (C_r) i.e $C_d >> C_r$. Therefore equation (1) can be reduced to

$$\frac{dQ}{dt} = P_s C_d$$

Hence the rate of skin permeation (dQ/dt) becomes constant if the C_d values remain fairly constant throughout the course of skin permeation. To maintain the C_d at a constant value, it is necessary to deliver the drug at a rate R_d that is either constant or always greater than the rate of skin absorption R_a ; i.e., $R_d >> R_a$. By doing so, the drug concentration on the skin surface C_d is maintained at a level equal to or greater than the equilibrium (or saturation) solubility of the drug in the stratum corneum C_s i.e., $C_d \ge C_s$ and a maximum rate of skin permeation (dQ/dt)_m, as expressed by equation, is thus achieved:

$$\left(\frac{dQ}{dt}\right)_m = P_s C_s$$

Thus, the magnitude of $(dQ/dt)_m$ is determined by the skin permeability coefficient of the drug (P_s) and its equilibrium solubility in the stratum corneum (C_s) . This indicates that skin permeation of drugs is stratum corneum limited.

2.3.6 Factors affecting transdermal permeation

In the development of transdermal drug delivery system and the subsequent transport of the drug(s) through the skin, a series of inter-related elements must be taken into consideration. These factors can be classified into four basic areas.⁴⁰

Skin structure and its properties

In respect of drug permeation, the most important tissue in this complex organ is the SC or horny layer, with usually provides the rate-limiting or slowest step in the penetration process.

Skin possesses several properties that regulate the rate of drug permeation.

These are:

Lipid bilayer

The lipid film that is present on the surface of the skin acts as a protective layer, which prevents the removal of moisture from the skin and helps in maintaining the barrier function of the SC.

Skin temperature

Increase in the skin temperature results in increase in the rate of skin permeation due to rise in solubility of drug in skin tissues and increased dilation of skin vessels.

Skin hydration

Hydration of SC can enhance transdermal permeability. Skin hydration can be achieved simply by covering or occluding the skin with plastic sheeting leading to accumulation of sweat and condensed water vapor.

Cutaneous drug metabolism

Catabolic enzymes present in the viable epidermis may render a drug inactive by metabolism and thus affect the topical availability of the drug. Other properties that effect drug penetration through the skin are the integrity and thickness of the SC, density of sweat glands and follicles and the presence of injuries on the skin, which leads to increase in the permeability of the drug.

2.3.7 Permeation enhancement

The penetrating molecule and its physical and chemical properties

- Water-soluble electrolytes like sodium chloride, potassium chloride and non electrolytes like glucose, urea, etc., penetrate the skin freely.
- Lipid soluble substances have the best chance of diffusing into the horny layer, especially when they are non-polar and/or moderately low molecular weight.
- Polymers and macromolecules of high molecular weight, like proteins and polysaccharides penetrate poorly.
- Partition coefficient: Drugs possessing both lipid and water solubility are favorably absorbed through the skin. Transdermal permeability coefficient shows a linear dependency on partition coefficient. A lipidwater partition coefficient of 1 or greater is generally required for optimal transdermal permeation.
- pH conditions: Application of solutions whose pH values are either very high or very low can be damaging to the skin. The flux of ionizable drugs can be affected by changes in pH that alter the ratio of charged and uncharged species and their transdermal permeability.

Penetrant concentration: Assuming membrane limited transport, increasing concentration of dissolved drug in the delivery system causes a proportional increase in its flux. Stability, binding affinity and vehicle effects are the other factors affecting drug permeation.

Effect of drug delivery system

The composition of drug delivery system not only affects the rate or drug release, but also the permeability of stratum corneum by means of hydration. The barrier permeability can be enhanced or decreased by using penetration enhancers or by addition of other solvents for example, benzocaine permeation was decreased by addition of polyethylene glycols of low molecular weight.

The release rate of the drug from the delivery system depends upon:

- The solubility of drug in the vehicle, i.e. whether the drug molecules are dissolved or suspended in the delivery system.
- The interfacial partition coefficient of the drug from the delivery system to the skin tissue.
- pH of the vehicle.

Effect of skin and formulation components

Although the process of penetrating the skin by drug has been simplified by representing it as one of simple unidirectional transport, in practice, the sequence is more complicated. Factors contributing include:

- Lack of homogeneity of the stratified tissues.
- The interruption of the stratum corneum, by hair follicles and sweat glands.
- The division of basal cells, their transport through the horny layer and their loss from the surface.
- Since drugs penetrate the skin under dynamic conditions, the medicament, vehicle components and occlusive hydration effects may progressively change the skin barrier.
- Sweat, sebum and cellular debris may enter the product, changing its physico-chemical characteristics.
- Volatile solvents may evaporate which will alter the chemical potential of the drug and may even develop supersaturated solutions.

Optimization of percutaneous absorption

One of the major problems in delivering a drug into or through the skin is its inherent impermeability. The barrier properties of the skin give rise to a skin bioavailability of only a few percent for many conventional topical products. It would be desirable to produce formulations with significantly greater bioavailability. This may result from rational and optimized formulation design, the use of appropriate penetration enhancers and/or the use of physical methods for penetration enhancement viz. electrical or ultrasound. 41, 42

Chemical penetration enhancers

Some solvents can remove lipids from the SC. The barrier function is reduced when the lipids are modified in this way, although the effect has been shown to be reversible. Some transdermal products contain high concentrations of solvents such as ethanol that may be capable of altering the lipid content of the skin. 43 Kunta et. al., in 1997 studied the effect of four terpenes: 1-menthol, d-limonene, carvacrol and linalool on percutaneous absorption of propranolol hydrochloride across excised hairless mouse skin. 44 The terpenes were very effective in promoting the skin transport of propranolol. A significant concentration effect was observed only with linalool. The permeation of propranolol across mouse skin from the hydrogel patch formulation containing 1-menthol was significantly higher than that from control.

An inspection of Fick's first law of diffusion shows that two major effects can be obtained if a formulation excipient permeates into the SC. It may intercalate into the structured lipids of the skin where it can disrupt the packing. The effect may render them more fluid thereby increasing the diffusion coefficient of the permeant. This has been demonstrated using differential scanning calorimetry (DSC) and measuring the effect on phase transition temperature, ⁴⁵ ESR studies ⁴⁶ and FTIR ⁴⁷ investigation.

The molecular characteristic that typifies an enhancer that disrupts the chain lipids is a polar head group with a long alkyl chain. (C_{10} to C_{14} appear optimal).^{48,49}

Compounds such as the nonionic surfactants have such properties and Brij 36T has been shown to be effective enhancer.⁵⁰ Oleic acid also acts by disrupting the skin lipid but appear to form pools in the lipids rather than being distributed homogeneously.⁵¹

The second way in which excipients can modify skin permeability is to shift the solubility parameter of the skin in the direction of that of the permeant. The solubility of the permeate in the outer layers of the skin will be increased and this in turn, improves the flux. Simple solvent type molecules such as propylene glycol, ethanol, transcutol[®] and N-methyl pyrollidone are thought to act in this way. For example, it is well known that propylene glycol permeates the skin, therefore, it must be distributed in the SC.⁵²

The mechanism of permeation through skin is a partition diffusion process and follows the Fick's law of diffusion. The major barrier to the drug penetration is the SC. The factors that affect drug release include the physicochemical properties of the vehicles (e.g. viscosity) and the solubilities of the drug and the vehicle. The interaction of the vehicle with the SC varies from simply occluding the skin to extracting lipid components from SC.⁵³

One way to extend the range of drugs, which may be administered transdermally, is to incorporate penetration enhancers into formulations. The safest and most widely used penetration enhancer is water. Increased hydration reduces the barrier property of skin.⁵⁴

Azone was the first molecule specifically designed as a skin penetration enhancer; for enhancing the skin transport of both hydrophilic and lipophilic drugs by reducing the order of the intercellular lipids. Metronidazole has an enhanced permeability if skin is pretreated with propylene glycol. If enhancement strategies include both an effect on diffusion and an effect on the solubility, a multiplicative result is predicated. Synergy between these approaches has been shown for numerous systems including metronidazole (Azone plus propylene glycol). 55

The effect is also possible when super saturation is combined with a lipid fluidizer e.g. for flurbiprofen (increased degree of saturation plus oleic acid). ⁵⁶

Terpenes have an excellent potential to be used as permeation enhancers. ⁵⁷

The three essential oils (eucalyptus, peppermint and turpentine oil) were found to increase the permeation of 5- fluorouracil through excised rat skin. Eucalyptus oil was found to be most active, causing 60 fold increase, while peppermint and turpentine oil showed 48 and 28 fold increase respectively. Mode of action of these enhancers may be due to a combined process of partition and diffusion, the latter being dominant.

Permeation enhancer d-limonene (20%) was found effective in enhancing the permeation of ketoprofen significantly.⁵⁸

The penetration index of piroxicam from piroxicam gel after one hour pretreatment with 10% cardamom oil in alcohol/pH 7.4 buffer was 34.9 times higher than that from untreated rabbit abdominal skin.

Further studies revealed that the increase in permeation was due to the presence of the monoterpenes, terpineol and acetylterpineol.⁵⁹

N-methyl-2-pyrrolidone (NMP) has been widely used to enhance the skin absorption of ibuprofen and flurbiprofen.⁶⁰ By the use of NMP, the flux of the ibuprofen increased 16 times and that of flurbiprofen increased 3 times through cadaver skin.

Goodman and Barry (1988) studied the effect of 2% azone in propylene glycol (PG) on the permeation of 5-fluorouracil (5-FU). Azones promoted the absorption of 5-FU by almost 100 fold, but in combination with Tween 20, the effect was less pronounced.⁶¹

The enhancing effect of pyrollidone derivatives on the transdermal penetration of 5-FU, triamcinolone acetonide, indomethacin and flurbiprofen were studied by Sasaki et al., 1991. It was reported that pyrollidone derivatives enhance the flux of penetrants in skin by increasing their solubility in the SC.⁶²

Chi et al., 1995, investigated the effect of fatty acids and urea in PG vehicle on the skin permeation of flurbiprofen through rat skin. The mixture of oleic

acid and urea showed a significantly higher permeation rate than oleic acid alone.⁶³

Ghosh et al., 1992, studied the comparison between hairless mouse and human cadaver skin and effect of n-decylmethyl sulfoxide on the permeability of metoprolol tartrate. Skin permeation rate across human cadaver skin was found to be lower than that of hairless mouse.⁶⁴

Stott et al., 2001, studied the transdermal permeation of propranolol through human skin in the presence of fatty acid (lauric, capric) penetration enhancers. There was no clear difference in permeation rates of the fatty acids compared.⁶⁵

Reddy et al., 1992, conducted the pharmacokinetic and pharmacodynamic studies of piroxicam gel after oral and transdermal administration to healthy male rats. They reported the improved effect of β -cyclodextrin on bioavailability and anti-inflammatory activity of piroxicam.

The mode of action of penetration enhancer in general is complex. At clinically acceptable concentration most enhancer interacts with the intercellular lipid domain of the SC.⁶⁷ The penetration enhancer increases the permeability either by fluidization, polarity alteration and phase separation or lipid extraction of the intercellular lipid bi-layer.⁶⁸

Turpentine oil was employed to enhance the dermal delivery of flurbiprofen, propylene glycol (PG):isopropyl alcohol (IPA) (30:70%, v/v) was used as vehicle for hydrogel formulation. ⁶⁹

The influence of isopropyl myristate and glyceryl monocaprylate on permeation of pentazocine was evaluated by *in-vitro* studies across rat skin.⁷⁰

Properties of penetration enhancers

The desirable properties of the penetration enhancers are as follows:

- They should be non-toxic, non-irritating and non-allergic.
- They should ideally work rapidly, the activity and duration of effect should be both predictable and reproducible.
- They should have no pharmacological activity in the body i.e. should not bind to the receptor sites.
- The penetration enhancer should work unidirectionaly i.e. should allow therapeutic agent into the body whilst preventing the loss of the endogenous materials from the body.
- When removed from skin the barrier properties should return both rapidly and fully.
- The penetration enhancer should be appropriate for formulation into diverse topical preparation, thus should be compatible with both the excipients and drugs.
- They should be cosmetically acceptable with an appropriate skin feel.

- The onset of the penetrating action of enhancer should be immediate.
- The enhancers should be odorless, colorless and economical so as to facilitate its use in the large scale commercial manufacture of transdermal drug delivery system.

Penetration enhancers may be incorporated into formulation in order to improve drug flux through diverse membranes. Diffusion through skin is controlled by the outermost layer, the SC, can be regarded as diffusion through the passive membranes. The steady state flux (J_{ss}) , of a drug through skin, can be approximated by Fick's first law of diffusion.

 $\delta C/\delta T = D\delta^2 C/\delta X^2$

Where C is the concentration of the diffusing substance, X the space coordinate measured normal to the section, D the diffusion coefficients and T is time.

Potential mechanisms of action of enhancers are varied and ranges from direct effect on the skin to modification of the formulation (indirect effect).⁷¹

Thus directly acting on the skin enhancers can:

- Act on the SC, intracellular keratin, denature it or modify its conformation causing swelling and increased hydration.
- Affects the desmosomes that maintains cohesion between cornecytes.
- Modify the intercellular lipid domain to reduce the barrier resistance of the bilayer lipids.

Alters the solvent nature of the SC to modify partioning of the drug or a cosolvent into the tissue.

The penetration enhancer can act indirectly by:

- Modification of the thermodynamic activity of the vehicle.
- Solvent permeating through the membrane could drag the permeant with it.
- Solubilising the permeant in the donor (e.g. Surfactants) especially where solubility is very low as with steroids in aqueous donor solution can reduce depletion effects and prolong drug permeation.

Formulation based approach

Different formulation and technologies have been attempted by researchers in order to deliver drug ranging from low molecular weight inorganic ions to high molecular weight polypeptides and oligonucleotides.

Lipid vesicles

Mixtures of propylene glycol (PG) and nonionic surfactants with sodium diclofenac (DFC) were prepared in the presence of phosphatidylcholine (PC) as transmembrane transport enhancers.⁷²

Diclofenac sodium containing lipogelosome formulations was evaluated and found that it possesses anti-inflammatory efficacy.⁷³

Proniosomes

Non-ionic surfactants Span 20, Span 40, Span 60 and Span 80 with and without cholesterol at percentages ranging from 0% to 50% were formulated and evaluated for effect of surfactant type and cholesterol content on drug release.⁷⁴

Niosomes

Celecoxib loaded niosomes were prepared and characterized *in-vitro*, ex-vivo and in vivo. The results of organ localization study showed that niosomal gel provided 6.5 times higher drug deposition as compared to carbopol gel.⁷⁵

Microemulsion

Isopropyl Myristate (IPM), Ethyl Oleate (EO) as oils, Aerosol OT as surfactant and sorbitan monooleate as cosurfactant were studied for potential to deliver flurbiprofen transdermally from microemulsions. Flurbiprofen showed higher *in-vitro* permeation from IPM as compared to that of from EO microemulsion.⁷⁶

Delivery of diclofenac sodium from microemulsion containing soybean oil, nonionic surfactants (Brij 58 and Span 80) and different alcohols (ethanol, isopropyl alcohol and propanol) as cosurfactant was attempted, with propanol producing maximum permeation.⁷⁷

Microemulsions (o/w) were prepared by the spontaneous emulsification method. Oleic acid was chosen as the oil phase, Labrasol S and Transcutol P

were used as surfactant and cosurfactant for transdermal delivery of terbinafine. ⁷⁸

Gel

Tenoxicam 1% w/w was formulated using 2% w/w hydroxy propyl guar derivative and 3% w/w sodium carboxy methyl cellulose as gelling agents. Rheological investigation revealed that hydroxy propyl guar derivative in 2% w/w strength resulted in gels with a higher pseudoplastic index.⁷⁹

Inclusion complex

Liposomes-encapsulated indomethacin/cyclodextrins (IMC/CD) inclusion complexes were formulated and high drug entrapment was achieved, as compared to conventional liposomes. Encapsulating IMC/CD inclusion complexes into liposomes resulted in a slow release of drug.⁸⁰

Iontophoresis

Transdermal permeation of methotrexate loaded into polyacrylamide-based hydrogel patch, across mice skin was studied *in-vitro* after pretreatment with terpenes and ethanol, alone or in combination with iontophoresis (DC/mDC).⁸¹

Iontophoresis of eight tripeptides, of the general structure alanine-X-alanine, was measured across hairless mouse skin *in-vitro* at constant current of 0.36 mA/cm², using Ag/AgCl electrodes.⁸²

Microneedles

High molecular weight molecules are problematic to deliver by passive diffusion and a novel technique which employs microneedles has been attempted for insulin delivery. 83

Organogels

Bovine serum albumin (BSA) and haemagglutinin (HA) were used as model antigens were delivered using complex gels, prepared by the addition of a hot aqueous niosome suspension (vehicle/water) to the sol phase (organic solution of the gelator) which resulted into a vesicle-in-water-in-oil (v/w/o) emulsion which cooled to an opaque, semi-solid, thermoreversible organogel.⁸⁴

Ultrasound

Pulsed ultrasound (1 MHz) was employed for transdermal permeation of indomethacin from an ointment across rat skin. Ultrasound energy applied for 10 to 19 min at intensities range of 1.0 to 2.5 Wcm⁻² was found to be effective.⁸⁵

Abrasion

The *in-vitro* permeation of acyclovir through human epidermal membrane using a rotating brush abrasion device was compared with acyclovir delivery using iontophoresis. It was found that application of brush treatment for 10 s at a pressure of 300 Nm⁻² was comparable to 10 min of iontophoresis. The observed enhancement of permeability observed using the rotating brush was

a result of disruption of the cells of the stratum corneum, causing a reduction of the barrier function of the skin. 86

Skin clearing agent was delivered into the tissue by rubbing it with fine 220 grit sandpaper. Gentle rubbing causes abrasion of the superficial skin layer including the stratum corneum, which otherwise prevents the skin clearing agents from freely diffusing into skin.⁸⁷

2.3.8 Determining skin permeation

The *in-vitro* skin permeation studies form different transdermal delivery systems can be evaluated using a two compartment diffusion cells. One compartment contains the drug component and the other contains a receptor solution. Excised skin specimen separates the two compartments with SC side facing the donor compartment.

The Frantz diffusion cell⁸⁸ and modified Keshary Chien cells⁸⁹ are the most commonly used diffusion cells to perform *in-vitro* permeation studies.

The skin permeation studies are performed by withdrawing the samples at predetermined time intervals from the side arm sampling port and assaying the drug concentration in the samples by a sensitive analytical method An equal amount of receptor phase is added into the receptor compartment to maintain uniform volume during the experiment.

A number of animal models have been used to perform *in-vitro* skin permeation studies. Ideally human skin is the most appropriate membrane for

performing *in-vitro* permeation studies.^{90,91} Due to the scarcity and difficulty in correlating the gender, race, age and skin condition of the donor, a number of animal models such as rat, hairless mouse, shed skin etc.^{92,93,94,95} have been used as possible models for human skin. The criterion for selection should be the correlation between permeation rate using human skin and animal skin. Synthetic membranes such as polydimethylsiloxane cellulose acetate, polyurethane have also been used to study *in-vitro* permeation release, kinetics, however they cannot predict accurately the permeabilities through the human skin.^{50,96}

The receptor solution used in diffusion cells should accept permeating drugs and provide water, biochemical and ions needed for the skin membrane to function in the permeation experiment at the proper pH and osmotic strength. Typically aqueous phases are used with polar drugs and ethanolic phases with lipophilic drugs. It is important that the concentration of the penetrant in the receptor solution remains low at all times, to prevent significant back diffusion. In general the thermodynamic activity should never exceed 10% of that in the donor formulation, in order to maintain an adequate diffusion gradient.

Other important factors that can effect *in-vitro* skin permeation are temperature, solubility and stirring.^{90,98} Control of receptor solution temperature is important to minimize variations in experimental conditions.

The temperature should be kept at normal physiological conditions, since temperature elevation may lead to increased hydration of the skin. It is known that a rise of 10°C in temperature can produce a 2 to 3 fold increase in permeation. Solubility and stirring are important to allow the permeant to be taken up and transported away from the skin after it has passed through, avoiding a concentration build up within or below the skin. Stirring of the receptor solution is also important to provide a homogeneous receptor solution. The concentration of the permeant in the receptor solution should remain low (less than 10%) compared with its solubility in the solution. If the permeant is relatively insoluble, solubility enhancing components can be added to receptor solution, however their effects on the skin must be considered.

2.4 THERAPEUTIC APPLICATIONS OF NSAID's

2.4.1 Inflammation

In 35 A.D., Celcus described the cardinal signs of inflammation-rubor et tumor cum calore et dolore - with Rudolph Virchows 19th century addition of functio laesa. These signs (of redness, swelling, heat, pain and loss of function) represent the outward signs of complex vascular, immunological and cellular reactions involving many soluble mediators of inflammation. This pathological phenomenon is essentially beneficial since it is the bodies

response to insult or injury and it serves to minimize the deleterious effects of a threatening agent by diluting, localizing, destroying and if possible, removing it. The process of inflammation can be initiated in any vascularized part of the body. It is a series of events, rather than a single occurrence and it follows a course that is generally uniform in its sequence but it may vary in intensity and duration, depending on the type and degree of initial stimulus. Inflammation is mediated by vasoactive amines (e.g. histamine and serotonin), cytokines, and leukocyte products (e.g. enzymes and reactive oxygen species), and the product of arachidonic acid metabolism (eicosanoids), all of which are of cellular origin. Inflammation is also regulated by the kinins, complement and clotting systems found in plasma.

2.4.2 Pain

Pain is an aversive sensory experience which elicits protective motor actions, results in learned avoidance and may modify species-specific behavior. Pain processing involves the perception and integration of noxious stimuli at specialized peripheral nerve endings called nociceptors. Once stimulated in the periphery, these specialized receptors transmit signals via the spinal cord to the somatosensory cortex of the brain for interpretation of the aversive stimulus. Tissue injury leads to nociception by direct mechanical and thermal damage to nerve endings, to inflammation by the release of chemicals and enzymes from damaged tissue and to pain generated by algogenic substances

and sprouting of damaged nerves into the injured tissue. 99,100 The same tissue injury promotes the release of substance P from nerve endings, resulting in vasodilatation and an increase in vascular permeability. 101 This causes local edema and in combination with the release of algogenic substances (e.g. prostaglandins, leukotrienes, bradykinin, serotonin and histamine), leads to inflammation and sensitization of nociceptors, resulting in pain. Thus, the primary afferent neuron serves a dual function: (a) transmission of neural stimuli and, (b) peripheral release of inflammatory mediators at the site of trauma. Primary afferent nociceptors are generally categorized as either myelinated (A-fibers) or unmyelinated (C-fibers). Normally, myelinated Afibers respond to noxious mechanical and thermal stimuli with a conduction velocity of 5-55 m/s and are responsible for initial stabbing-like pain. Unmyelinated C- fibers, have a slower conduction velocity of 0.8 m/s, respond to a wider range of noxious stimuli including mechanical, thermal and chemical. These afferent nociceptors are responsible for the chronic pain sensation, referred to as burning or aching pain. 102,103 It is likely that in the vast majority of pain conditions, whether inflammatory or neuropathic, there is an associated phase of inflammation in which a variety of chemical mediators are able to alter the functions of primary afferent neuron. Although the majority of nociceptors respond to thermal and mechanical stimulation, chemical signaling is likely to be the most common and diverse form of signal generation in all types of fine afferent fibers. ¹⁰⁴

2.4.3 Rheumatoid arthritis

Rheumatic diseases have affected mankind since ages and are one of the commonest inflammatory conditions in developing countries. Rheumatoid arthritis forms a major prototype of rheumatic disease and is a common cause of disability. It is a chronic systemic inflammatory disorder that may affect many tissues and organs e.g. skin, blood vessels, heart, lungs, and muscles-but principally attack the joints, producing a nonsupportive proliferative synovitis that often progresses to destruction of the articulator cartilage and ankylosis of the joints. Although the etiopathogenesis of rheumatoid arthritis remains uncertain, it is currently believed that it involves diverse and complex factors such as genetic background, rheumatic factor circulating antibodies, free oxygen radical, etc. 105 Hence the exposure of an immunogentically susceptible host to an anthropogenic microbial antigen triggers the initial acute arthritis. But it is the continuing auto immune reaction, the activation of CD₄+ helper T cells and the local release of inflammatory mediators and cytokines that ultimately destroys the joint.

About 10% of the world's population is afflicted by rheumatoid arthritis, women three to five times more often than men. The peak incidence is in the twenties and forties but no age is immune. 106,107

2.4.4 Osteoarthritis

Also known as degenerative joint disease, the term osteoarthritis implies an inflammatory disorder, in which, the inflammatory cells maybe present. The disease is considered to be an intrinsic one, affecting the cartilage-particularly the articular cartilage - in which biochemical and metabolic alterations results in its breakdown. Osteoarthritis joins heart disease and cancer as one of the dividends of growing older. In the great majority of instances, it appears insidiously, without apparent initiating cause, as an aging phenomenon (idiopathic or primary osteoarthritis). In these cases, the disease is usually oligoarticular (affects few joints) but may be generalized. In about 5% of cases, osteoarthritis may appear in younger individuals having some predisposing conditions, such as previous macro traumatic or repeated micro traumatic injuries to joint; a congenital developmental deformity or underlying systemic disease such as diabetes, ochronosis, hemochromatosis, or obesity. In these settings the disease is called secondary osteoarthritis. Gender has some influence on distribution. The knees and hands are more commonly affected in women and the hips in men. Chondrocytes play a primary role in the process and constitute the cellular basis of the disease. Other mediators, such as prostaglandin derivatives and IL-6, also have a role in the process of matrix degradation. 108

2.4.5 Role of prostaglandins

Prostaglandins (PG's) are members of a large family of arachidonic acid oxygenation products. Eicosanoids (20 carbon fatty acids) are derived from naturally occurring eicosapolyenoic acids, of which arachidonic acid is the most common. Arachidonic acid, which is either obtained from the diet or synthesized from linoleic acid, is widely distributed in the body and is usually stored covalently bound in its esterified form, in the phospholipid fraction of the cell membranes of most body cells. The initiation of PG synthesis occurs when cells are stimulated to release arachidonic acid by the triggering of specific receptors or by less specific activation elicited by mechanical stress. This, in turn, increases intracellular calcium levels which facilitate the translocation of the enzyme phospholipase A2 or C from the cytoplasm to binding sites on cell membranes. After release, arachidonic acid is then reesterified or metabolized by cyclo-oxygenase (COX) enzyme to produce oxygenated products such as PGs and TXs. Cyclo-oxygenase products include PGD₂, PGE₂, PGF₂, and PGI₂ and TXA₂. 109

Prostaglandin E₂ has received a great deal of attention in recent years because of the many effects it exerts on the physiology of mammals. A large body of evidence now points to the existence of high affinity PGE-specific binding sites in a wide range of tissues. Such binding sites have been identified in adipocytes, endothelium, bronchioles, bone, adrenal gland, corpora lutea,

myometrium, kidney, intestinal epithelium, liver, heart, brain and platelets. Not only is PGE₂ a participant in numerous homeostatic responses, but as a prostanoid it plays an important role in both acute and chronic inflammation. Prostaglandin E₂ is produced in large quantities in inflammatory conditions, whether these are induced by mechanical trauma, corrosive chemicals, burning, radiation, antigen-antibody reactions or immune-mediated responses. Prostaglandin E₂ may be generated from a variety of inflammatory cells, (e.g. neutrophils and eosinophils), but macrophages appear to be the richest source.¹¹⁰

Prostaglandin E₂ is a potent vasodilator and high local concentrations of PGE₂ in inflammatory conditions are sufficient to induce local hyperemia through vasodilatation. On its own, PGE₂ is a poor inducer of edema, but it can enhance the edema-producing properties of agents such as bradykinin and histamine.¹¹¹ The E series PGs have hyperalgesic activity thus, while they are not pain-producing in their own right, they sensitize pain receptors to stimulation by agents such as bradykinin and serotonin. It is this ability of PGs to amplify the pain-producing effects of other agents which explains the analgesic action of the non-steroidal anti- inflammatory drugs (NSAIDs).¹¹²

2.4.6 Clinical approach

The conventional drug treatment of arthritis involves two primary goals first, the relief of pain, which is often the presenting symptom and the major complaint of the patient and second, the slowing of the tissue damaging process.

Reduction of inflammation with nonsteroidal anti-inflammatory drugs often results in relief of pain for significant period. Furthermore, most of the nonopioid analgesics (aspirin, etc.) have anti-inflammatory effects and are appropriate for the treatment of both acute and chronic inflammatory conditions.

The glucocorticoids (prednisone, etc.) also have powerful anti-inflammatory effect and when first introduced were considered to be the ultimate solution to the treatment of inflammatory arthritis through the inhibition of phospholipase A₂ the enzyme responsible for the liberation of arachidonic acid from membrane lipids and the selective inhibition of the expression of COX-2 enzyme. Unfortunately, the toxicity associated with chronic corticosteroid therapy (Fractures, infections, cataracts, etc.) inhibits their use except in the control of acute flare-up of joint disease.

Another important group of agents are characterized as slow-acting antirheumatic drugs (SAARDs) or disease modifying antirheumatic drugs (DMARDs).

These drugs might arrest or slow the progression of bone and cartilage destruction by modifying the disease itself. The effects of disease modifying therapies may take 6 weeks to 6 months to become evident, i.e., they are slow

acting compared with NSAIDs. These therapies include methotrexate, azathioprine, penicillamine, hydroxy chloroquine and chloroquine, organic gold compounds, etc. unfortunately, they may also be more toxic than the NSAID's. Therefore, the NSAID's have assumed a major role in the treatment of rheumatic diseases particularly rheumatoid arthritis and osteoarthritis.

2.4.7 Non-steroidal anti inflammatory drugs (NSAID's)

The oxygenation of polyunsaturated fatty acids such as arachidonic acid, by the cyclooxygenase (COX) enzyme or lipoxygenase enzyme systems leads to the formation of several important biologically active lipids of the inflammatory process, including the prostaglandins (PGs), thromboxane (TX) and prostacyclin and leukotrienes¹¹⁵ (Fig. 3).

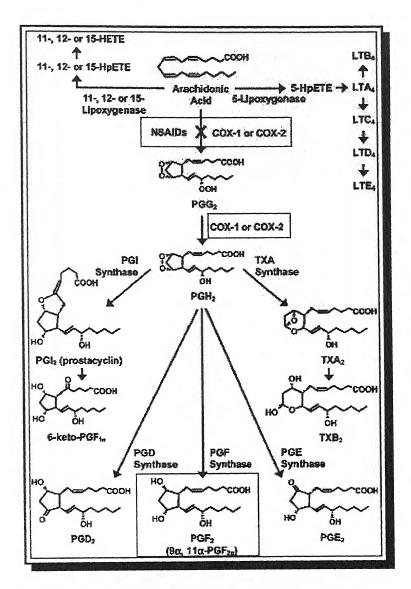


Figure 3 - Prostaglandin and thromboxane synthesis pathway.

These compounds, known collectively as the eicosanoids, have a vital role in the initiation and maintenance of several components of inflammation including, but not limited to, vasodilation, increased vascular permeability, leukotaxis, pain, bronco constriction and platelet aggregation. In 1971, John

Vane discovered that aspirin and indomethacin inhibited the biosynthesis and hence the release of stable PGs from tissue homogenates. 116 The finding that PG production was suppressed or abolished while the release of the upstream phospholipase enzyme was unaffected implied that NSAIDs acted by preventing the conversion of arachidonic acid to PG. Vane, in 1994, subsequently proposed that cyclo-oxygenase (COX) inhibition may be the common denominator for all therapeutic and side effects of NSAIDs. 117 Presently, two isofoms of COX are known to be present in eukaryotic organisms: COX-1 and COX-2. Non-steroidal anti-inflammatory drugs have an affinity for the ubiquitous COX-1 enzyme which is expressed in most tissue types other than mature erythrocytes. Cyclo-oxygenase-1 is involved in cellular homeostasis since it synthesizes PGs in response to physiological stimuli at a rate proportional to the availability of its substrate, arachidonic acid. 118 The COX-1 enzymes active site is a long hydrophobic channel which was discovered through X-ray crystallography. 119 It is likely that aspirin-like drugs inhibit PG formation by excluding arachidonic acid from the upper portion of the channel. Cyclo-oxygenase-2 which shares approximately 60% homology with COX-1 is expressed or induced at sites of inflammation through the action of cytokines, growth factors, tumor promoters and bacterial lipopolysaccharide. Macrophages and other migratory inflammatory cells have abundant COX-2 activity. 120 The long term therapy with non specific NSAID's (which inhibit both COX; COX-I and COX -2) is frequently limited by their adverse effects, particularly those caused by erosion of gastric mucosal protection ranging form dyspepsia to life threatening perforation, ulcers, obstructions and bleed.¹²¹

Transdermal delivery affords an improved approach to the administration of drug by maintaining therapeutic but constant concentration of drug in the blood for a desired period of time, usually between 1 and 7 days.

OBJECTIVE
AND
PLAN OF WORK

3. OBJECTIVE AND PLAN OF WORK

3.1 AIM OF RESEARCH

- TO SELECT THE APPROPRIATE DRUG CANDIDATE
- TO IDENTIFY THE MOST POTENTIAL DRUG DELIVEY
 SYSTEM
- FORMULATION OF DRUG DELIVEY SYSTEM
- TO EXPLORE THE EFFECT OF VARIOUS ADDITIVES ON THE FORMULATION
- CHARACTERIZATION AND EVALUATION OF THE CHOSEN
 DRUG DELIVEY SYSTEM
- TO STUDY THE EFFICACY OF THE DRUG DELIVEY SYSTEM
 THROUGH ANIMAL EXPERIMENTATION

3.2 OBJECTIVE OF STUDY

THE STUDY WAS ALSO DESIGNED TO ACHIEVE THE FOLLOWING GOALS:

- TO DEVELOP A STABLE, REPRODUCIBLE AND PATIENT NON-FRINGING DRUG DELIVERY SYSTEM FOR USE IN PAIN AND INFLAMMATION.
- EXTENDED DURATION OF ACTIVITY THAT ALLOWS GREATER PATIENT COMPLIANCE OWING TO ELIMINATION OF MULTIPLE DOSING SCHEDULES.
- TO OBVIATE SPECIFIC PROBLEMS RELATED TO THE ORAL ABSORPTION OF THE DRUG.
- TO OBVIATE SPECIFIC PROBLEMS ASSOCIATED WITH THE DRUGS E.G. GASTRO-INTESTINAL TRACT IRRITATION, LOW ABSORPTION, FIRST PASS EFFECT, FORMATION OF METABOLITES THAT CAUSE SIDE, EFFECTS AND SHORT HALF-LIFE NECESSITATING FREQUENT DOSING.

3.3 RATIONALE OF THE STUDY

Although NSAIDs have potent pharmacological activities with oral administration it also has unwanted problems such as first pass metabolism and GI irritation. Considering the fact that NSAIDs are usually used for long period it is desirable to reduce these side effects. One of the recent efforts to eliminate some of the problems of the traditional dosage forms is the transdermal drug delivery system.

3.3.1 Hydrogel based transdermal drug delivery of ketorolac

Ketorolac (administered as tromethamine salt), a prostaglandin synthetase inhibitor is a nonsteroidal anti-inflammatory drug with potent analgesic and moderate anti-inflammatory activities. ¹²² Ketorolac has been studied clinically and its efficacy and safety as an analgesic in postoperative pain and cancer has been established. Administered as oral and injectable formulations, it has shown high analgesic potency almost equivalent to that of morphine. ¹²³ Unlike narcotic analgesics, ketorolac does not alter gastric motility or hemodynamic variables or adversely affect respiration, nor is it associated with abuse or addiction potential as in case of narcotic analgesics. Ketorolac therefore, is a relatively favorable therapeutic agent for the management of moderate to severe pain. ¹²⁴

Ketorolac thus has substantial clinical potential and developing an alternative dosage form that is easy to administer; is painless; non invasive; easy to comply and avoids first pass metabolism, is worthwhile. The transdermal route encompasses all the above advantages.

Despite these advantages, only a limited number of drugs can be administered percutaneously, due to low skin permeability of most drugs through the skin. The penetration through stratum corneum is the rate-limiting step for delivery of most of the drugs. To overcome this problem, vehicles, 125 penetration enhancers, 126 ultra sound, 127 and electro-transport facilitated 128 systems have been attempted in the development of a transdermal delivery system of ketorolac. Prodrug approach has also been investigated for enhanced dermal delivery. However, the most widely used technique involves use of chemical penetration enhancers or solvents that modify the thermodynamic activity.

3.3.2 Microemulsion based gel for transdermal delivery of dexamethasone

Microemulsion is defined as a dispersion consisting of oil, surfactant, cosurfactant and aqueous phase, which is a single optically isotropic and thermodynamically stable liquid solution with a droplet diameter usually within the range of 10-100 nm. 131 Microemulsions have several advantages such as enhanced drug solubility, good thermodynamic stability, enhancing effect on transdermal ability over conventional formulations. 132 There are several permeation enhancement mechanisms of microemulsions such as an increased concentration gradient and thermodynamic activity toward skin and the permeation enhancement activity of the components of microemulsions. 133 So far, much attention has been focused on the dermal delivery of drugs such as estradiol and lidocaine using microemulsions. 134 Recently, different hydrogel matrices such as carbomer 934 and carrageenan have been used to increase the viscosity of microemulsion for dermal application. 135,136,137 The addition of hydrogel matrix into the microemulsion resulted in the formation of the microemulsion based hydrogel, which is more suitable for dermal application when compared with microemulsion.

Dexamethasone, a synthetic glucocorticoid, 138 is widely used in inflammatory condition and to suppress normal immune response. 140 It is used as a therapeutic agent in alcohol withdrawal syndrome, 141 cerebral oedema, 142

congenital adrenal hyperplasia, 138, nausea and vomiting specially associated with high dose of anticancer agents, 143 high altitude disorder, 142 cerebral malaria, opportunistic mycobacterial infections, respiratory disorders, skin disorders, 138 rheumatism. 144 Dexamethasone possesses most of the desirable physicochemical and biological properties, e.g. half-life of 2 and 5 h, plasma protein binding nearly about 67%, a small dose ranging from 0.5 to 9 mg daily, hepatic first pass effect of the drug and gastric irritation upon oral administration, 145 to be evaluated for transdermal application. A number of researchers have also reported methodologies for increasing the transdermal absorption of drugs from formulation by employing: deformable carriers, 146 transfersomes, 147 patches, 148 ocular microemulsion, 149 iontophoresis, 150,151,152 and phonophoresis. 153,154

3.3.3 Proniosomal based transdermal drug delivery of piroxicam

Colloidal particulate carriers such as liposomes¹⁵⁵ or niosomes¹⁵⁶ have been widely employed in drug delivery systems and producing them from proniosomes provides them a distinctive advantage. These carriers can act as drug reservoirs, the rate of drug release can be controlled by modification of their composition. These lipid vesicles can carry both hydrophilic drugs (by encapsulation) and hydrophobic drugs (in lipid domain). Due of their capability to carry a variety of drugs, these lipid vesicles have been extensively used as, drug delivery systems¹⁵⁷ targeting of drug,¹⁵⁸ controlled release¹⁵⁹ or permeation enhancement.¹⁶⁰ But there remains certain draw backs to be addressed and can be avoided if they are prepared in dry form. Proniosomes, prepared in dry form and hydrated by agitation in hot water to form niosomes provide an alternative with prospective for drug delivery via the transdermal route.^{161,162}

The transdermal route of drug delivery has many advantages for administration of drugs in local and systemic therapy. But, skin is widely recognized for its effective barrier properties compared with other biological membranes. The low permeability of the skin makes it a minor port of entry for drugs. The vesicular drug delivery is thus potentially beneficial as vesicles tend to fuse and adhere to the cell surface, this is believed to increase the

thermodynamic activity gradient of the drug at vesicle-stratum corneum interface thus leading to enhanced permeation rate.

Piroxicam, a non-steroidal anti-inflammatory drug, is indicated in rheumatoid arthritis, osteoarthritis and a variety of other acute and chronic musculoskeletal disorders, dysmenorrhea and as ordinary analgesics. ¹⁶³ However, the use of piroxicam has been associated with a number of gastrointestinal disorders. ¹⁶⁴ Dermal delivery is an alternative route, but requires a formulation which ensures the deep skin penetration. Various formulations based approaches were made to deliver piroxicam viz organogel based formulation was attempted by Agrawal et. al., 2004, ¹⁶⁵ bucal delivery by Attia et. al., in 2004, ¹⁶⁶ mucoadhesive system by Cilurzo et. al., 2005, ¹⁶⁷ microspheres based drug delivery ^{168,169,170} by Raman et.al., 2005, Berkland et. al., 2004, Georgeta et. al., 2004, iontophoresis ¹⁷¹ was attempted by Curdy et. al., 2001, cyclodextrin based enhancement ¹⁷² was carried out Murthy et. al., 2004 and gel based formulation by Shin et. al., 2000¹⁷³ and Santoyo et. al., 1995. ¹⁷⁴

3.4 CHOICE OF THE DRUG

Transdermal delivery systems have been developed for local and systemic administration of drugs. Relationship between skin permeability and physicochemical properties of drugs have been investigated to clarify the mechanisms of skin permeation, as well as to predict plasma drug concentrations after applying transdermal delivery.

Skin permeation consists of sequential physical and biological processes. The partition and diffusion of drugs are recognized as important physical processes. Diffusion in skin is determined by the molecular weight of drug. Smaller molecules permeate the skin more rapidly than larger molecules.

The influence of partition can be evaluated by correlating skin permeability with drug lipophilicity such as the logarithm of n-octanol / buffer partition coefficient.

Passive transdermal absorption often exhibits a parabolic relationship with the maximum at intermediate lipophilicity, which is explained by a change in the rate limiting step for skin permeation. For hydrophilic drugs the partition into stratum corneum is the rate limiting step of skin permeation, whereas for lipophilic drugs the partition between stratum corneum and viable epidermis becomes important. Some generalizations on chemical structure of drugs and their skin permeation can be made:

- Water-soluble electrolytes like sodium chloride, potassium chloride and non electrolytes like glucose, urea etc. penetrates the skin freely.
- Lipid soluble substances can diffuse into the horny layer, especially when they are non polar and of moderately low molecular weight.
- Polymers and macromolecules of high molecular weight like proteins and polysaccharides penetrate poorly.

For putative selection of a drug candidate for transdermal delivery there are some basic prerequisites pertaining to the physicochemical and pharmacokinetic properties of the drug molecule, namely, it should have low molecular weight, low dose, balanced oil-water partition coefficient, low melting point, relatively short biological half-life, require repeated dosing, require long term treatment, tend to cause gastrointestinal irritation, non irritant to skin, undergo rapid first pass metabolism and require better patient compliance.

The following drugs were selected as a potential candidate for drug delivery because

3.4.1 Ketorolac

Ketorolac has been selected as a drug candidate for transdermal delivery because

- It has a low molecular weight i.e. 376.40
- It has a melting point of 162 to 164°C

- It has a plasma half life of $5.3 \pm 1.2 \text{ h}$.
- It causes unwanted side effects such as systemic complications and gastric irritation.
- It is used for periods ranging from one day to five days.
- It undergoes hepatic first pass metabolism.
- It shows incompatibility when mixed with other drugs.

3.4.2 Dexamethasone

Dexamethasone has been selected as a drug candidate for transdermal delivery because

- It has a low molecular weight i.e. 392.45
- It has a melting point of 268° to 272° C
- It has a plasma half life of 3 to 4.5 h.
- It causes unwanted side effects such as systemic complications and gastric irritation.
- It is used for long period.
- It undergoes hepatic first pass metabolism.
- It has a lipophilic character.

3.4.3 Piroxicam

Piroxicam has been selected as a drug candidate for transdermal delivery because

- It has a low molecular weight i.e. 331.35
- It has a melting point of 198°C
- It has a plasma half life of 50 h.
- It causes unwanted side effects such as systemic complications and gastric irritation.
- It is used for long period.
- It undergoes hepatic first pass metabolism.
- It has a lipophilic character.

3.5 DRUG PROFILE

3.5.1 Ketorolac (tromethamine salt)

Structural representation of ketorolac

IUPAC name: (±)-5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-

carboxylic acid, with 2-amino-2-

(hydroxymethyl)-1,3-propanediol (1:1)

Molecular weight: 376.40

Category: Pyrrolo-pyrrole group of nonsteroidal anti-

inflammatory drugs (NSAIDs)

Description: White/off white powdered solid. a racemic

mixture of [-]S and [+]R ketorolac tromethamine. Ketorolac tromethamine may exist in three crystal forms. All forms are equally soluble in water. Ketorolac tromethamine has a pKa of 3.5 and an n-

octanol/water partition coefficient of 0.26.

Indications:

Oral, injection: Short-term (≤ days) management of moderately-severe acute pain requiring analgesia at the opioid level.

Ophthalmic: Temporary relief of ocular itching due to seasonal allergic conjunctivitis; postoperative inflammation following cataract extraction; reduction of ocular pain and photophobia following incisional refractive surgery, reduction of ocular pain, burning and stinging following corneal refractive surgery.

Dental: Oral, injection: Short-term (≤ days) management of moderately-severe acute pain requiring analgesia at the opioid level.

Pharmacokinetics

Distribution: Poor penetration into CSF; crosses placenta;

enters breast milk

Protein binding: 99%

Metabolism: Hepatic

Half-life elimination: 2-8 h; prolonged 30% to 50% in elderly

Excretion: Urine (61% as unchanged drug)

Dose: Oral – Adult: 10-20 mg initially then 10 mg

every 4-6 h. Not more than 40 mg daily.

Parenteral - Adult: 10 mg I.V. or I.M. followed

by 10-30 mg every 4-6 h. Maximum daily dose

90 mg.

Dosage:

Tablet: 10 mg

Injection: 1 ml (30 mg/ml)

Ocular drops: 5 ml (0.5%)

3.4.2 Dexamethasone

Structural representation of dexamethasone

IUPAC name: 9α -fluoro-11β, 17 α, 21-trihydroxy-16 α

methylpregna-1, 4-diene-3, 20-dione.

Molecular formula: $C_{22}H_{29}FO_5$

Molecular weight: 392.47

Category: Adrenocortical steroid (Anti-inflammatory)

Description: White or almost white crystals or a crystalline

powder, odorless, melting point 268□-272□C

Solubility: Sparingly soluble in ethanol (95%), in acetone,

and in methanol, slightly soluble in chloroform,

very slightly soluble in ether. Practically insoluble in water.

Indications:

Rheumatic disorders: As adjunctive therapy for short-term administration in acute gouty arthritis, acute rheumatic carditis, ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis, including juvenile rheumatoid arthritis

Pharmacokinetics

Protein binding:

77%

Metabolism:

Metabolized in the liver by CYP3A4.

Half-life elimination:

1.8 to 3.5 h.

Excretion:

Up to 65% of a dose is excreted in urine within

24 h

Dose:

Adult- initial 0.5 to 20 mg I.V./ I.M. repeated as

required. Total dose not to exceed 80 mg.

Children – 200 to 500 mcg/kg body weight

I.V./I.M.

Dosage:

Tablet: 0.5 mg

Injection: 2 ml, 5 ml, 10 ml and 30 ml (4

mg/ml)

Drops: 10 ml (0.5 mg/ml)

3.5.3 Piroxicam

Structural representation of piroxicam

IUPAC name: 4-hydroxyl-2-methyl- N -2-pyridinyl-2 H -1,2-

benzothiazine-3-carboxamide 1,1-dioxide.

Molecular formula: $C_{15}H_{13}N_3O_4S$

Molecular weight: 331.35

Description: Piroxicam occurs as a white crystalline solid,

sparingly soluble in water, dilute acid and most

organic solvents. It is slightly soluble in alcohol

and in aqueous solutions. It exhibits a weakly

acidic 4-hydroxy proton (pKa 5.1) and weakly

basic pyridyl nitrogen (pKa 1.8).

Indications: Osteoarthritis, rheumatoid arthritis.

Pharmacokinetics

Distribution: Excreted in breast milk.

Protein binding:

99%

Metabolism:

In the liver by hydroxylation; no active

metabolites.

Half-life elimination:

50 h

Excretion:

Eliminated primarily in the urine, small amount

in feces; 5% excreted unchanged.

Dose:

Adult: 10 to 30 mg daily. In case of acute

disorders 40 mg daily as single or divided doses

for 7-14 days.

Dosage:

Tablet:

10 mg

Capsule:

10 mg, 20 mg

Injection:

1 ml, 2 ml (20 mg/ml)

2 ml (40 mg/ml)

3.6 EXCIPIENT PROFILE

Almond oil

BIOLOGICAL SOURCE: *Prunus dulcis*, syn. *Prunus amygdalus*, *Amygdalus communis*, *Amygdalus dulcis* is a species of Prunus belonging to the subfamily Prunoideae of the family Rosaceae.

DESCRIPTION: It is a mild, lightweight oil, rich in unsaturated fats and essential fatty acids with a slight odour and a nutty taste.

SOLUBILITY: It is almost insoluble in alcohol but readily soluble in chloroform or ether.

Butanol

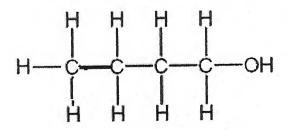
SYNONYMS: Butyl alcohol

CHEMICAL NAME: 1- Butanol

EMPIRICAL FORMULA: C₄H₁₀O

MOLECULAR WEIGHT: 74.11

STRUCTURAL FORMULA:



FUNCTIONAL CATEGORY: Solvent

DESCRIPTION: Clear, colourless liquid

SOLUBILITY: Moderately miscible with water

Carbopol 934

SYNONYMS: Carbomer

CHEMICAL NAME: Carbomer

EMPIRICAL FORMULA: (C₃H₄O₂)_n

MOLECULAR WEIGHT: 7x10⁵ to 4x10⁹

STRUCTURAL FORMULA:

FUNCTIONAL CATEGORY: Bioadhesive, emulsifying agent, release modifying agent, suspending agent, tablet binder, viscosity enhancer.

DESCRIPTION: White coloured fluffy, acidic, hygroscopic powders with slight characteristic odour.

SOLUBILITY: Soluble in water and after neutralization in ethanol (95%) and glycerin.

Chloroform

SYNONYMS:

CHEMICAL NAME: Trichloromethane

EMPIRICAL FORMULA: CHCl₃

MOLECULAR WEIGHT: 119.38

STRUCTURAL FORMULA:

FUNCTIONAL CATEGORY: Reagent, solvent, anesthetic

DESCRIPTION: Colorless liquid with characteristic odour.

SOLUBILITY: 0.8 g/100 ml at 20 °C in water

Cholesterol

SYNONYMS: Cholestrin

CHEMICAL NAME: Cholest-5-en-3β-ol

EMPIRICAL FORMULA: C₂₇H₄₆O

MOLECULAR WEIGHT: 386.67

STRUCTURAL FORMULA:

FUNCTIONAL CATEGORY: Emollient, emulsifying agent

DESCRIPTION: White or faintly yellow, almost odourless, powder or granules.

SOLUBILITY: Soluble in acetone and vegetable oil. Practically insoluble in water.

Dimethyl sulfoxide

SYNONYMS: Methylsulfinylmethane

CHEMICAL NAME: Dimethyl sulfoxide

EMPIRICAL FORMULA: C2H6OS

MOLECULAR WEIGHT: 78.13

STRUCTURAL FORMULA:

FUNCTIONAL CATEGORY: Solvents, Cryoprotectant

DESCRIPTION: It is a clear, colorless hygroscopic liquid. When it is pure it has little odour, but impure samples smell strongly of dimethyl sulfide.

SOLUBILITY: It is readily soluble into a wide range of organic solvents such as alcohols, esters, ketones, chlorinated solvents and aromatic hydrocarbons. It is also miscible in all proportions with water.

d-limonene

SYNONYMS: 4-Isopropenyl-1-methylcyclohexene

CHEMICAL NAME: 1-Methyl-4-prop-1-en-2-yl-cyclohexene

EMPIRICAL FORMULA: C₁₀H₁₆

MOLECULAR WEIGHT: 136.24

STRUCTURAL FORMULA:

DESCRIPTION: Limonene is a hydrocarbon, classified as a cyclic terpene. It is a colourless liquid at room temperatures with an extremely strong smell of oranges. It takes its name from the lemon, as the rind of the lemon, like other citrus fruits, contains considerable amounts of this chemical compound, which is responsible the smell. Limonene is a chiral molecule and as is common with such forms, biological sources produce one enantiomer: the principal

industrial source, citrus fruit, contains D-limonene ((+)-limonene), is the (R)-enantiomer.

Egg lecithin

SYNONYMS: Egg yolk lecithin

CHEMICAL NAME: Lecithin

STRUCTURAL FORMULA: α-Phosphatidylcholine

FUNCTIONAL CATEGORY: Emollient, emulsifying agent, solubilizing agent

DESCRIPTION: Physical form may vary from viscous liquids to powders and colour from brown to light yellow.

SOLUBILITY: Soluble in aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, mineral oils, fatty acids. Practically insoluble in polar solvents and water.

Ethanol

SYNONYMS: Ethyl Alcohol; ethyl hydroxide

CHEMICAL NAME: Ethanol.

EMPIRICAL FORMULA: C₂H₆O.

MOLECULAR WEIGHT: 46.07

STRUCTURAL FORMULA:

FUNCTIONAL CATEGORY: Antimicrobial preservatives, disinfectants, skin penetrant, solvent.

DESCRIPTION: Ethanol is a clear, colorless, mobile and volatile liquid with a slight, characteristic odor and burning taste.

SOLUBILITY: Miscible with chloroform, ether, glycerin and water.

Eucalyptus oil

STRUCTURAL FORMULA: Cineole

BIOLOGICAL SOURCE: Eucalyptus oil is obtained from the leaves and branches of the Eucalyptus tree, *Eucalyptus maculate*, *E.polybractea*, *E.Smithic*, *E.globules or E. australiana*.

DESCRIPTION: It is colorless or pale yellow liquid with an aromatic and campharceous odour.

CHEMICAL CONSTITUENTS: The most important constituents are eucalyptol (cineol) present in *E. globules* up to 70% of its volume. It consists chiefly of terpene (cymene).

Hydroxypropylmethyl cellulose (HPMC)

SYNONYMS: Hypromellose, Culminal, Methocel, Pharmacoat, Tearsol, Metolose, Cellulose.

CHEMICAL NAME: Cellulose, 2-hydroxypropyl methyl ether.

EMPIRICAL FORMULA: $C_8 H_{15} O_6$ - $(C_{10} H_{18} O_6)_n$ - $C_8 H_{15} O_5$

MOLECULAR WEIGHT: Appox. 15,000

STRUCTURAL FORMULA:

FUNCTIONAL CATEGORY: Gel former

DESCRIPTION: An odorless, tasteless, white or off-white, practically odorless, hygroscopic fibrous powder or granules.

SOLUBILITY: Soluble in cold water and produces a clear to opalescent, viscous colloidal mixture, practically insoluble in chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol/methanol and dichloromethane.

Isopropyl alcohol

SYNONYMS: Isopropanol

CHEMICAL NAME: Propan-2-ol

EMPIRICAL FORMULA: C₃H₈O

MOLECULAR WEIGHT: 60.10

STRUCTURAL FORMULA:



FUNCTIONAL CATEGORY: Antimicrobial preservatives; disinfectants; skin penetrant; solvent

DESCRIPTION: Isopropyl alcohol is a clear, colorless, volatile, flammable liquid with a characteristic, spirituous odor resembling that of a mixture of ethanol and acetone; it has a slightly bitter taste.

SOLUBILITY: Fully miscible in water

Linseed oil

SYNONYMS: Flax seed oil (contains chiefly linolenic acid)

CHEMICAL NAME: cis, cis, cis-9,12,15-Octadecatrienoic acid

EMPIRICAL FORMULA: C₁₈H₃₀O₂

MOLECULAR WEIGHT: 278.43

STRUCTURAL FORMULA: Linolenic acid

HO 1
$$\frac{9}{9}$$
 $\frac{6}{12}$ $\frac{3}{15}$ $\frac{0}{18}$

DESCRIPTION: It is a clear to yellowish drying oil derived from the dried ripe seeds of the flax plant (*Linum usitatissimum*, Linaceae). It is obtained by pressing, followed by an optional stage of solvent extraction.

Maltodextrin

SYNONYMS: Lycatab

CHEMICAL NAME: Maltodextrin

EMPIRICAL FORMULA: (C₆H₁₀O₅)_n. H₂O

MOLECULAR WEIGHT: 900 - 9000

STRUCTURAL FORMULA:

FUNCTIONAL CATEGORY: Coating agent, diluent for tablet and capsule, binder, viscosity increasing agent.

DESCRIPTION: Non sweet, odourless, white powder or granules.

SOLUBILITY: Freely soluble in water, slightly soluble in ethanol (95%).

Nutmeg oil

BIOLOGICAL SOURCE: Nutmeg oil is obtained by the steam distillation of ground nutmeg belonging to the genus Myristica e.g., Myristica argentea, Myristica fragrans, Myristica inutilis, Myristica malabarica, Myristica macrophylla, Myristica otoba, Myristica platysperma etc.

CHEMICAL CONSTITUENT: Nutmeg oil is a volatile oil containing borneol and eugenol.

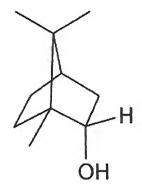
CHEMICAL NAME: Borneol - endo-1, 7, 7-Trimethyl- bicyclo[2.2.1]heptan-

2-ol

EMPIRICAL FORMULA: C₁₀H₁₈O

MOLECULAR WEIGHT: 154.25

STRUCTURAL FORMULA: Borneol



DESCRIPTION: The oil is colorless or light yellow and smells and tastes of nutmeg. Nutmeg oil is a volatile oil containing borneol and eugenol.

Olive oil

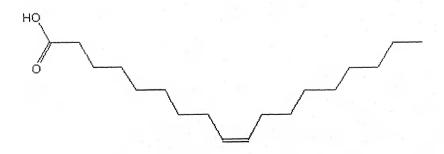
SYNONYM: Salad oil, sweet oil.

BIOLOGICAL SOURCE: it is obtained by cold expression from ripe fruits of *Olea europoea* linn.

FAMILY: Oleaceae.

DESCRIPTION: It is a pale yellow liquid which some time greenish tint, with slight odor and bland taste.

STRUCTURAL FORMULA: Oelic acid



CHEMICAL CONSTITUENTS: Olive oil is a monounsaturated fat and, unlike butter, lard, and other fats, contains a large proportion of easily digested fats and no cholesterol. Olive oil contains mixed glycerides of oleic acid (56-85%), linoleic acid (3.5-20.0%), palmitic acid (7.5-20%) and stearic acid (0.5-5%). There is also limit of sterol. It also contains volatile alcohol (hexanol, e-2-hexanol, 2-3 hexanol), aldehyde and acetylated ester.

n-Pentanol

CHEMICAL NAME: Pentan-1-ol

EMPIRICAL FORMULA: C₅H₁₂O

MOLECULAR WEIGHT: 88.15

STRUCTURAL FORMULA:

OH

FUNCTIONAL CATEGORY: Solvent

DESCRIPTION: A colorless liquid with an unpleasant aroma.

SOLUBILITY: 2.7 g/100ml in water

Polyethylene glycol-400

SYNONYMS: Carbowax, Lutrol E; PEG

CHEMICAL NAME: α -Hydro- ω -hydroxy poly (oxy-1, 2-ethanedilyl)

EMPIRICAL FORMULA: H (OCH2CH2) n OH

MOLECULAR WEIGHT: 380 - 420

STRUCTURAL FORMULA:

FUNCTIONAL CATEGORY: Ointment base, plasticizer, solvent, suppository base, tablet and capsule lubricant.

DESCRIPTION: It is occur as clear, colorless or slightly yellow colored, viscous liquids. SOLUBILITY: Liquid polyethylene glycol is soluble in acetone, alcohols, benzene, glycerin and glycols

n- Propanol

SYNONYMS: Propanol

CHEMICAL NAME: 1-Propanol

EMPIRICAL FORMULA: C₃H₈O

MOLECULAR WEIGHT: 60.1

STRUCTURAL FORMULA:

FUNCTIONAL CATEGORY: Disinfectant, solvent

DESCRIPTION: Colorless, flammable chemical compound with a strong odor

SOLUBILITY: Miscible with benzene, chloroform, ethanol (95%), ether, glycerin and water. Soluble in acetone; insoluble in salt solutions.

Sorbitan ester

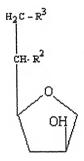
SYNONYMS: Sorbitan monostearate

CHEMICAL NAME: Sorbitan mono-octadecanoate.

EMPIRICAL FORMULA: C₂₄H₄₆O₆

MOLECULAR WEIGHT: 431.0

STRUCTURAL FORMULA:



FUNCTIONAL CATEGORY: Emulsifying agent, non ionic surfactant, solubilizing agent, wetting and dispersing / suspending agent

DESCRIPTION: Sorbitan esters occur as solid with distinctive odour and taste.

SOLUBILITY: Sorbitan esters are generally soluble or dispersible in oils; they are also soluble in most organic solvents. In water, although insoluble, they are generally dispersible.

Sorbitol

SYNONYMS: D-sorbitol

CHEMICAL NAME: D-Glucitol

EMPIRICAL FORMULA: C₆H₁₄0₆

MOLECULAR WEIGHT: 182.17

STRUCTURAL FORMULA:

FUNCTIONAL CATEGORY: Humectant, plasticizer, sweetening agent, tablet and capsule diluent.

DESCRIPTION: Odourless, white or almost colourless, crystalline, hygroscopic powder

SOLUBILITY: Practically insoluble in chloroform and ether, slightly soluble in methanol.

Transcutol

SYNONYMS: diethylene glycol monoethyl ether

CHEMICAL NAME: 2-(2-Ethoxyethoxy) ethanol or 3,6-dioxa-1-octanol

EMPIRICAL FORMULA: C₆H₁₄O₃.

MOLECULAR WEIGHT: 134.17

STRUCTURAL FORMULA:

FUNCTIONAL CATEGORY: Industrial solvent

DESCRIPTION: It is a clear, colorless, hygroscopic liquid. It is flammable.

SOLUBILITY: Soluble in low molecular weight alcohols

EXPERIMENTAL

HYDROGEL BASED TRANSDERMAL DRUG DELIVERY SYSTEM OF KETOROLAC

4. EXPERIMENTAL - HYDROGEL BASED TRANSDERMAL DRUG DELIVERY SYSTEM OF KETOROLAC

4.1 MATERIALS

S.No.	Name of Ingredient	Source	
1	Ketorolac	Ranbaxy Laboratories,	
		Devas, India.	
2	Hydroxy propyl methyl cellulose i.e.	Colorcon Asia Pvt. Ltd.,	
2	HPMC (Methocel® K15M)	Goa	
3	Ethanol	E. Merck (India) Ltd,	
		Mumbai.	
	n- propanol, isopropyl alcohol, n- butanol,	Central Drug House,	
4	n-pentanol and propylene glycol, dimethyl sulphoxide (DMSO) and Eucalyptus oil	New Delhi.	
_	d-limonene	Hi Media Labs,	
5		Mumbai.	
	Diethylene glycol monoethyl ether (Transcutol® P)	Gattefosse, France	
6		through Colorcon Asia	
		Pvt. Ltd., Goa.	

4.2 CHARACTERIZATION OF KETOROLAC

4.2.1 Physical characteristics of ketorolac (tromethamine salt)

Nature : Powdered solid

Colour : White - off white

Odour : Odourless

Taste : Bitter

Melting point : $164 \pm 1^{\circ}$ C

Solubility : 14.46 ± 0.53 mg/ml

pH (1 in 100 solution). $: 6.7 \pm 0.4$

4.2.2 Identification test

I.R. spectroscopic studies

Infra red (I.R.) study was carried out on FTIR 840 Shimadzu, Japan. Potassium bromide was used for preparing the sample for I.R. spectroscopic study. The spectrum (Fig. 4) was recorded using the software IRsolution Ver.1.21 in the scanning range of 400-2000 nm and resolution of 2 cm⁻¹. Peaks corresponding to various functional groups are reported in Table 2.

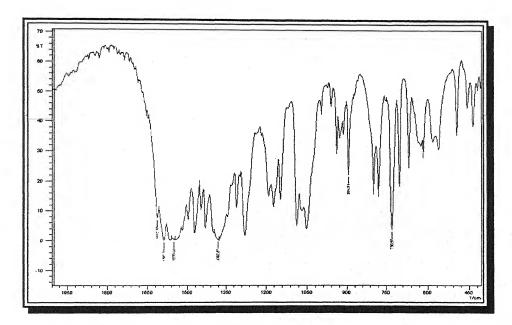


Figure 4 - I.R. spectrum of ketorolac sample

Table 2 - Peaks in I.R. spectrum indicating various functional groups of ketorolac sample

S.No.	Functional group	Peak observed	
1	Aromatic C-H stretching	733.97	
2	Mono substituted phenyl ring	894.91	
3	Aliphatic C-H bending	1382.37	
4	C=O stretching	1550.66	
5	C=O stretching	1587.31	
6	C=O stretching band	1612.38	

4.2.3 Other tests

Loss on drying : On drying for 3 h at 60 °C loss in weight was $0.2\% \pm$

0.1% (As per U.S.P.: NMT 0.5% of its weight).

Residue on ignition : $0.05 \pm 0.03\%$ (As per U.S.P.: NMT 0.1% of it's

weight)

4.2.4 Identification of impurities

To 100 ml of water and 100 ml of dichloromethane in a 250 ml separator was added 30 mg of Ketorolac Tromethamine sample and 1 ml of 1N hydrochloric acid. It was shaken and the layers were allowed to separate. 1.0 ml of the dichloromethane layer was transferred to a vial and evaporated to dryness. To it 1.0 ml of solvent mixture [water and tetrahydrofuran (70:30)] was added. Chromatographic system - The liquid chromatographic study was carried out using Shimadzu LC-20 AT system, Shimadzu Corporation, Kyoto, Japan with UV spectrophotometeric detection (20-SPD) at 313 nm and Phenomenex

Luna C-18 column (250×4.6 mm, 5μm, California, USA) maintained at a constant temperature of about 40°C. The flow rate was adjusted to 1 ml/min. Mobile phase used was mixture of buffer solution (5.75g of monobasic ammonium phosphate in 1000 ml of water and adjusted to a pH of 3.0 with phosphoric acid) and tetrahydrofuran (70:30). The drug solution was injected and the peak responses were recorded. The presence/absence of impurities or degradation products if present shows peaks at the relative retention times of about 0.63 for the ketorolac 1-hydroxy analog and 0.89 for the ketorolac 1-keto analog in comparison to that of ketorolac. The chromatograph (Fig. 5) did not show presence of any such peaks related to the degradation product of ketorolac thus confirming its purity.

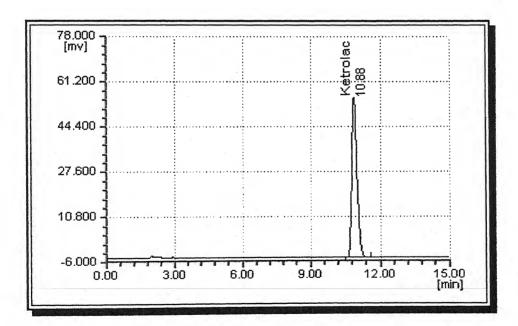


Figure 5 - HPLC chromatograph of ketorolac

Conclusion

On the basis of the above tests, it could be confirmed that the drug sample of ketorolac tromethamine is an authentic one and free of impurities/degradation products.

4.3 ANALYTICAL METHODOLOGY

4.3.1 U.V. absorption spectrometric studies

A 50 μ g/ml solution of ketorolac tromethamine was prepared in phosphate buffer saline (PBS) of pH 7.4 and scanned for U.V. absorption in the range of 200 to 400 nm. The U.V. absorption spectra (Fig. 6) exhibited a λ_{max} (wavelength of maximum absorbance) at 324 nm.

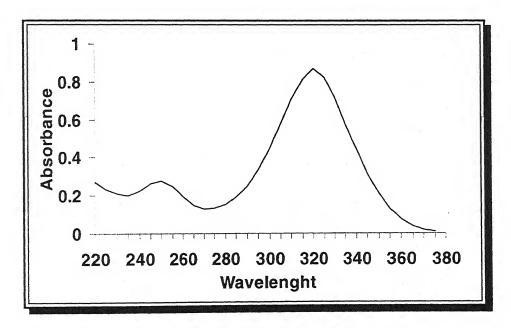


Figure 6 - U.V. absorption spectra of ketorolac tromethamine in PBS pH 7.4

Composition of buffer solution employed in experiments

The phosphate buffer saline (PBS) was prepared by dissolving the mentioned quantity of monobasic sodium phosphate dihydrate (NaH₂PO₄.2H₂O) and dibasic sodium phosphate heptahydrate (Na₂HPO₄.7H₂O) in 1000 ml of distilled water and adding sodium chloride to make a 20mM buffer solution (Table 3).

Table 3 - Composition of buffer solution employed in experiments

рН	Monobasic sodium phosphate monohydrate NaH ₂ PO ₄ .2H ₂ O (g)	Dibasic sodium phosphate heptahydrate Na ₂ HPO ₄ .7H ₂ O (g)	Distilled water q.s. to (ml)
5.4	1.334	0.089	1000
6.4	1.028	0.684	1000
7.4	0.312	2.075	1000

4.3.2 Comparison of UV absorption spectra before and after storage of ketorolac in PBS pH 7.4

Drug solution was prepared in PBS of pH 7.4 and divided into three portions as under:

- A. Portion scanned U.V. spectrophotometrically immediately after preparation of solution.
- B. Portion kept under laboratory conditions for 48 h after covering the container with aluminum foil.
- C. Portion kept under laboratory conditions and exposed to light for 48 h.

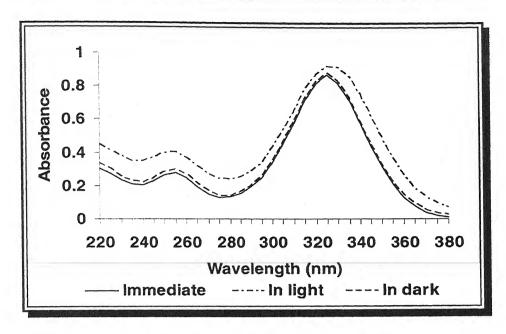


Figure 7 - U.V. absorption spectra of ketorolac tromethamine portions A - Portion scanned U.V. spectrophotometrically immediately after preparation of solution (Immediate)

B - Portion kept under laboratory conditions for 48 h after covering the container with aluminum foil (In dark)

C - Portion kept under laboratory conditions and exposed to light for 48h (in light)

As shown in the Fig. 7 apparently no change in the λ_{max} was observed when the solution was kept in dark while change was observed on storing the solution in light. It was therefore necessary to perform all experiments in dark or due protection from light be provided.

4.3.3 Calibration curve of ketorolac (tromethamine salt)

Stock solution of ketorolac tromethamine (ketorolac equivalent to 67.8%) was made by dissolving 100mg of drug in 100 ml of PBS pH 7.4. Serial dilutions in the range of 5-50 μ g/ml were made from the stock solution and absorbance

of these solutions were determined at the λ_{max} of 324 nm using PBS pH 7.4 as blank (Table 4).

Table 4 - Absorbance values of ketorolac in phosphate buffer saline pH 7.4 at $\lambda max~324~nm$

S.No.	Concentration of ketorolac tromethamine (mcg/ml)	Concentration of ketorolac (mcg/ml)	Mean absorbance	± S.D. (n=4)
1	5	3.39	0.094	0.0045
2	10	6.78	0.196	0.0040
3	20	13.56	0.387	0.0139
4	30	20.34	0.582	0.0239
5	40	27.12	0.777	0.0186
6	50	33.90	0.961	0.0500

The absorbance values corresponding to each concentration were then statistically evaluated and plotted as a standard graph, between absorbance on y-axis and concentration of ketorolac (μ g/ml) on x-axis. The calibration curve is shown in Fig. 8. The calibration curve followed Lambert's law in the selected concentration range.

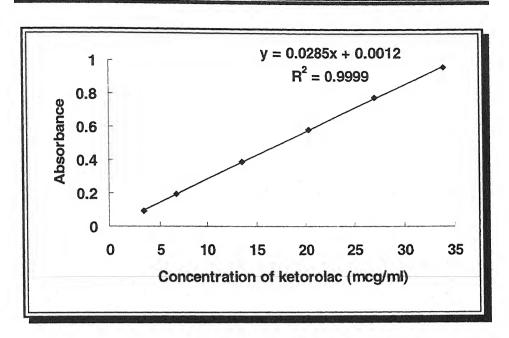


Figure 8 - Calibration curve of ketorolac in phosphate buffer saline of pH 7.4 at λmax of 324 nm

4.3.4 Method for measurement of ketorolac in permeation studies

One millileter sample of receptor medium was withdrawn at defined time intervals. The receptor media was replaced immediately with equal volume of fresh PBS of pH 7.4. The sample withdrawn was filtered through Whatmann filter paper no. 42 and the volume was made up. The samples were analyzed spectrophotometrically at 324 nm. The concentration of drug was read from the calibration curve prepared as reported in section 4.3.3. The cumulative amount of drug permeated was calculated by the following equation:

$$\frac{Drug \ permeated}{(mcg/cm^2)} = \frac{\frac{Correntration(mcg/ml)*Dilution \ factor*}{Volume \ of \ receiver \ compartment(ml)}}{Area \ of \ permeation(cm^2)} + \frac{Drug \ lost}{in \ sampling}$$

4.3.5 Analysis of drug content in the ketorolac gel formulation

Gel was collected into 30 ml capacity screw capped vials with Teflon lined caps and shaken with PBS pH 7.4 for 2 h to extract out the drug. The aqueous phase was collected, filtered and the quantity of drug determined UV spectrophotometrically at λ_{max} of 324 nm using the regression equation obtained for the calibration curve as reported in section 4.3.3.

Amount of drug
$$(mg) = \frac{Concentration (mcg / ml) \times Dilution factor}{1000}$$

Percentage drug content and percent recovery is shown in Table 5.

Table 5 - Drug content and percent recovery of ketorolac from transdermal patches

Formulation	% Drug content	% Recovery	
	/ Zing content	Mean	± S.D. (n=4)
pH 5.4	99.70	99.70	0.21
pH 6.4	99.81	99.81	0.18
pH 7.4	99.72	99.72	0.23
Ethanol	99.91	99.91	0.05
Propanol	99.27	99.27	0.47
IPA 10%	98.87	98.87	0.25
IPA 15%	98.94	98.94	0.30
IPA 20%	98.75	98.75	0.08
IPA 25%	99.66	99.66	0.16
n-Butanol	98.43	98.43	0.42
n-Pentanol	99.32	99.32	0.61
PG	98.56	98.56	0.53
Limonene	99.05	99.05	0.36
DMSO	99.44	99.44	0.48
Transcutol 5%	98.79	98.79	0.87
Eucalyptus oil 5%	98.11	98.11	0.70
Eucalyptus oil 7.5%	99.83	99.83	0.48
Eucalyptus oil 10%	98.88	98.88	0.11
Eucalyptus oil 15%	99.61	99.61	0.43

4.3.6 Analysis of stability study samples

Patch formulations stored in humidity chamber maintained at 40 $^{\circ}$ C / 75 $^{\circ}$ C relative humidity, were withdrawn at 0, 15, 30, 45, 60 and 90 days. The patch was peeled open, the contents withdrawn and analyzed.

4.4 PREPARATION OF KETOROLAC FORMULATION

4.4.1 Preparation of ketorolac gel system

Ketorolac gel system was prepared in buffer solution by dissolving ketorolac and adding HPMC (hydroxyl propyl methylcellulose) with continuous stirring so as to uniformly disperse the polymer. Alcohols and/or permeation enhancers were mixed with the vehicle before adding the polymer. The gel was kept overnight at ambient temperature in a tightly closed container to allow uniform gelling (cold dispersion method).

Various gel systems were formulated which comprised:

- A. drug, polymer and buffer solution
- B. drug, polymer, buffer solution and alcohols
- C. drug, polymer, buffer solution, alcohols and penetration enhancers

4.4.2 Fabrication of patch of ketorolac gel system

Transdermal patches (reservoir type) of ketorolac were fabricated by filling ketorolac gel preparation (0.25g/cm²) within a shallow compartment made of drug impermeable backing membrane and a hollow ring shaped device and a drug impermeable backing membrane (laminated aluminum foil). A micro porous adhesive tape of a larger area was stuck onto the impermeable backing membrane to bring the transdermal patch in intimate contact with the skin. The device was closed by a release liner on the open side (Fig. 9).

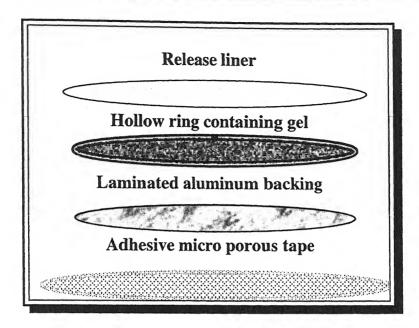


Figure 9 - Design of transdermal patch of ketorolac

The reservoir type transdermal delivery system of ketorolac comprised of four basic components:

- a. An adhesive backing to bring the patch in intimate contact with the skin and help the patch adhere to the skin.
- b. Drug impermeable backing membrane to avoid loss of solvent and/or formulation.
- c. Drug reservoir viscous gel preparation consisting of the therapeutic agent.
- d. Release liner which protects the integrity of the dosage form and is removed before the use of the transdermal system.

Adhesive backing

The adhesive backing consisted of micro porous adhesive tape commercially available by 3M, which was larger in area then the impermeable backing membrane.

Impermeable backing membrane

It consisted of a laminated aluminum foil available from local vendors and widely used in packing food containers.

4.4.3 Method of application of the transdermal system

The release liner was safely removed and the patch was securely placed over the hairless dorsal abdominal skin. The patch was brought in intimate contact with the skin by cautiously pressing the micro porous adhesive tape to the skin.

4.4.4 Preparation of abrasive gel

Distilled water was heated to 80°C and to it was added 10%w/w crushed seeds of apricot (*Prunus armeniaca*). The polymer was then added slowly and allowed to disperse uniformly (hot dispersion method). It was then cooled to form viscous gel into which was entrapped crushed apricot seed pieces.

4.4.5 Method of application of abrasive gel

One gram of abrasive gel was topically applied with the help of index finger on the depilated site before application of formulation and was gently

massaged onto the skin for a period of two minutes. The crushed apricot pieces were dusted off with soft cloth.

4.5 IN-VITRO SKIN PERMEATION STUDIES OF KETOROLAC GEL PREPARATIONS

4.5.1 Preparation of rat skin

Sprague-Dawley rats were used in the present study. The rats that had been sacrificed after other experimental work were used in the study; care was taken that only such animals be used whose skin was not affected in the experimental procedure. The dorsal skin of the rat was shaved with the help of hair clipper and full thickness skin was surgically removed. The subcutaneous fat of the skin was removed with a pair of scissors. The cleaned skin was wrapped in aluminum foil and stored in freezer at –21°C until further use. The skin was slowly brought to room temperature before using the same.

4.5.2 Fabrication of apparatus for *in-vitro* skin permeation studies

In-vitro skin permeation studies were carried out using modified Keshary Chien cell. The diffusion cells were fabricated locally by Rama Scientific Industries, Govindpuri, New Delhi. (Fig. 10). The material used for fabrication was type I borosilicate glass.

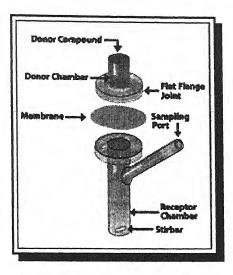


Figure 10 - Design of Keshary Chien diffusion cell used in *in-vitro* skin permeation studies

The assembly consisted of two chambers: a. the open upper chamber known as donor chamber and, b. the lower receptor chamber containing a sampling port and Teflon coated magnetic bead. The internal diameter of the recipient compartment was 1.6 cm. The capacity of the receiver chamber was 11 ml and the area of diffusion was 2.0 cm².

There were hooks on both upper and lower chamber for attachment of spring so that the two chambers could be securely attached. The receptor chamber was maintained at a temperature of $37 \pm 1^{\circ}$ C.

4.5.3 In-vitro skin permeation studies of ketorolac gel TDS preparations

In-vitro skin permeation studies were performed on a Keshary Chien diffusion cell with an effective diffusional area of 2.0 cm2 and 11 ml of receiver chamber capacity using rat abdominal skin. The skin was brought to room temperature and mounted between the donor and receiver compartment of the Keshary Chien diffusion cell, the stratum corneum side faced the donor compartment and the dermal side faced the receiver compartment. The assembly was covered with aluminum foil to protect it from light. Initially the donor compartment was empty and the receiver chamber was filled with phosphate-buffered saline (PBS) pH 7.4. The receiver fluid was stirred with a magnetic bead and the temperature was maintained at 37 ± 1 °C. The PBS was replaced aftrt every 30 minutes until the skin was equilibrated. The skin was found to completely stabilize in a maximum of 6 h as the receptor fluid showed negligible absorbance indicating complete stabilization of the skin. After complete stabilization of the skin, 0.5 g of gel was placed into donor compartment and sealed with Parafilm M[®] to provide occlusive conditions. Samples were withdrawn at regular intervals (2, 4, 6, 8, 10, 12, 20, 22, and 24h), and analyzed for drug content by UV spectrophotometer at λ_{max} of 324 nm using PBS pH 7.4 as blank as mentioned in section 4.3.4. The receptor volume was immediately replaced with fresh receptor medium. The sampling port was also covered with Parafilm M® to prevent evaporative loss of receptor medium.

4.5.4 Effect of pH on *in-vitro* skin permeation of ketorolac through rat skin

Ketorolac gel system was prepared which consisted of 2%w/w ketorolac and 2%w/w HPMC swelled in PBS solution of defined pH by cold dispersion method. Three different PBS solutions of pH 5.4, 6.4 and 7.4 were employed for preparing gel system.

4.5.5 Effect of various alcohols on *in-vitro* skin permeation of ketorolac

Ketorolac gel system was prepared which consisted of 2%w/w ketorolac and 2%w/w HPMC in PBS solution at optimized pH of 5.4 by cold dispersion method. Various alcohols viz. ethanol, n-propanol, IPA (isopropyl alcohol), n-pentanol, n-butanol and PG (propylene glycol) were evaluated at 10% w/w concentration. The selected alcohol was further optimized at 10%, 15%, 20% and 25%w/w concentration. Enhancement ratio was calculated by comparing the steady state flux of ketorolac in the presence and absence of alcohol across rat abdominal skin.

4.5.6. Effect of permeation enhancers on *in-vitro* skin permeation of ketorolac

Ketorolac gel system was prepared which consisted of 2%w/w ketorolac and 2%w/w HPMC (hydroxyl propyl methylcellulose) in buffer solution at pH of 5.4 along with IPA (isopropyl alcohol) at 25%w/w and permeation enhancers. The gel system for the reservoir compartment of the transdermal patch was prepared by cold dispersion method. Various enhancers viz. DMSO (dimethy sulfoxides), eucalyptus oil, d-limonene and transcutol® (diethylene glycol monoethyl ether) were added at 5% w/w concentration. The concentration of the enhancer screened out was further optimized at 7.5, 10 and 15%w/w. The effectiveness of the enhancer was determined by comparing the steady state flux of ketorolac in the presence and absence of enhancer.

4.5.7 Effect of abrasion of skin on *in-vitro* skin permeation of ketorolac

The permeation potential of dermal abrading agents was evaluated by applying abrading gel section 4.4.5. Followed by application of transdermal gel system consisting of ketorolac (2% w/w), HPMC (2%w/w), IPA (25%w/w) and eucalyptus oil (10%w/w) formulated using buffer solution of pH 5.4.

4.6 HISTOLOGICAL STUDIES

4.6.1 Effect of formulation on the histology of rat abdominal skin

Transdermal patch formulations were applied for 24 h on the dorsal surface of excised rat skin mounted on the diffusion cell. The patch was removed and the adhering formulation was wiped off with tissue paper. The skins were fixed in 10% v/v formalin solution in saline for at least 72 h before being further processed. The skin was sectioned vertically and each section was dehydrated and embedded in paraffin wax. The subdivided tissues were stained with hematoxylin and eosin. The sections were observed under microscope and photographed at 40 X magnification. Untreated skin served as control.

4.7 PHARMACOLOGICAL STUDIES

4.7.1 Animal studies

Animal experimentation was approved by the Institutional Animal Ethics Committee of Bundelkhand University (Reg. No. 716/02/a/CPCSEA). The reported experiments were carried out in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

4.7.2 Skin irritation study

As various preparations, when applied dermally, might elicit skin irritation therefore, to assess the skin sensitizing potential ketorolac transdermal patch was applied onto the dorsal skin of Wistar rats (220–250 g) of either sex. The animals were housed in polypropylene cages with free access to standard laboratory diet and water. They animals were acclimatized for at least seven days before experimentation. The dorsal abdominal skin of the rats were shaved 24 h prior to study and abraded before application of TDS. The site of application was occluded with gauze and covered with non-sensitizing microporous tape. After 24 h, the patch was removed and the score of erythema was determined by Drazie test¹⁷⁵ as follows: 0 – no erythema; 1 – very slight erythema (barely perceptible); 2 – well-defined erythema; 3 – moderate to severe erythema and 4 – severe erythema (beet redness) to slight eschar formation (injuries in depth).

4.7.3 Acetic acid induced writhing effect

Ketorolac was administered via oral (2mg/kg) and transdermal route 1h before administration of writhing agent. The transdermal patch was securely adhered over the depilated dorsal abdominal skin (9 cm²). The rats received intra peritoneally (i.p.) acetic acid (20 mg/kg, 2 ml/kg) in deionized water. The number of writhes (i.e., abdominal constriction followed by dorsiflexion and extension) occurring during a 15 min period beginning 15 min after acetic

acid administration was measured. The results are expressed as percentage inhibition in writhes.

4.7.4 Carrageenan induced paw edema

The anti-inflammatory efficacy was evaluated by carrageenan induced paw inflammation in Wistar rat (approx. 220-250g). The rats were assigned to treatment groups so that each group was weight balanced. Ketorolac was administered via oral (2mg/kg) and transdermal route 1h before carrageenan injection. The transdermal patch was securely adhered over the depilated dorsal abdominal skin (9 cm²). The rats received intraplantar injection of 50 µl of 0.5%w/v carrageenan suspension into the left hind paw subcutaneously by inserting the needle into the central part of the paw. The paw volume was measured and compared to with that found in animals treated with carrageenan alone. The right hind paw which served as control was treated with physiological saline solution. The inflammatory response was determined by measuring the changes in paw volumes with a screw gauge (Mitutoyo, Kanagawa, Japan) at 0, 2, 4, 6, 8 and 10 h after the carrageenan injection.

The degree of paw swelling was calculated as:

Swelling (%)=
$$\frac{Vt-V}{V}X100$$

where, V_1 is the volume of the carrageenan-treated paw, V is that of the non-treated paw.

Inibition (%) =
$$\frac{Sc - St}{Sc} X 100$$

where, Sc is the swelling of the control paw, St is that of the test formulation treated paw. The AUC (area under the curve) was determined by trapezoidal method.

4.8 DATA ANALYSIS

4.8.1 Calculation for predicting desired flux (K_0) required to be achieved for attaining effective plasma concentration

Using the equation:

$$AK_0 = C_p V_d K_{el}$$

The desired flux required to achieve the effective plasma concentration can be calculated from:

C_p, the therapeutic plasma concentration (ng/ml); = 900 ng/ml

 V_d , the volume of distribution: 14.7 L

 K_{el} , the elimination rate from plasma: 0.143 h^{-1}

and assuming that area of applicable system: 25 cm²

the target permeation rate (K_0) of the drug was found to be: 75.6 μ g/cm²/hr.

In order to achieve the desired C_p the formulation must achieve the calculated flux when using a patch of 25 cm². However, a patch of larger (max. 50 cm²) or smaller area can be employed.

4.8.2 Data analysis of in-vitro permeation studies

The *in-vitro* skin flux was determined from Fick's law of diffusion considering the transport of drugs across the skin barrier as a process of passive diffusion.

Various parameters were obtained using the equations reported below 176:

$$J_{SS} = \frac{(dM)}{dt} \cdot \frac{1}{A} = Kp \times \Delta C$$

and

$$K_p = \frac{J_{SS}}{C_o}$$

 J_{SS} is the skin flux ($\mu g/cm^2/h$), dM/dt is the amount of drug permeated per unit of time, A is the permeation area (cm^2), Kp is the permeability coefficient (cm h^{-1}), ΔC is the concentration gradient and C_0 is the donor phase concentration. In all the experiments, the concentrations in the donor cell remained constant, the receiver cell concentrations did not exceed 10% of the donor cell concentrations and ΔC was assumed to be equal to the donor cell concentrations.

The steady-state flux J_{SS} was determined from the slope of the linear portion of the cumulative amount permeated per unit area versus time plot. The lag time (T $_{lag}$) was determined by extrapolating the linear portion of the curve to the abscissa.

The Kp can also be determined as:

$$K_p = \frac{D x P}{h}$$

D is the diffusion coefficient of the drug into the stratum corneum which reflects the facility for the molecules to move through the membrane strata and is a function of the molecular structure of the diffusant. P is the partition coefficient of drug between stratum corneum and vehicle of formulation. h is the barrier thickness for skin (stratum corneum thickness, 15.4 μ m), assuming that the SC represents the main rate-limiting barrier. Partition coefficient was calculated from values of Kp and D while D was approximated indirectly from the lag time $(T_{lag})^{177}$

$$D = \frac{h^2}{6T_{lag}}$$

Enhancement ratio (ER) was calculated from the following equation:

Enhancement ratio (ER) =
$$\frac{J_{SS} \text{ of test formulation}}{J_{SS} \text{ of control formulation}}$$

4.8.3 Statistical analysis

All experiments were performed in quaduplicate and the data were expressed as the mean value \pm S.D. The results were analyzed by Student's t-test using Statistica for windows (version 5.0) from StatSoft, Inc., USA. The results were evaluated at probability level of 0.05.

EXPERIMENTAL

MICRO EMULSION GEL BASED
TRANSDERMAL
DELIVERY SYSTEM OF DEXAMETHASONE

5. EXPERIMENTAL - MICROEMULSION GEL BASED TRANSDERMAL DELIVERY SYSTEM OF DEXAMETHASONE

5.1 MATERIALS

S.No.	Name of Ingredient	Source		
1	Dexamethasone	Arbro Pharmaceuticals, New		
		Delhi, India.		
2	Almond oil	Fluka Chemicals Corp.,		
		Switzerland		
3	Linseed oil and nutmeg oil	Aldrich Chemicals Company,		
		USA		
4	Egg lecithin	HiMedia Laboratories, Mumbai		
5	Isopropyl alcohol (IPA)	Ranbaxy Chemicals Pvt. Ltd.,		
		New Delhi		
6	Olive oil and Carbopol 934	CDH Pvt. Ltd., New Delhi.		
7	Hydroxy propyl methyl cellulose	Colorcon Asia Pvt. Ltd.,		
	(HPMC K ₁₅ M)	Bangalore.		

5.2 CHARACTERIZATION OF DEXAMETHASONE

5.2.1 Physical characteristics of the drug

Nature : Crystalline powder

Colour : Almost white

Odour : Odourless

Taste : Bitter

Melting point : $263 \pm 1^{\circ}$ C

Solubility : 1 in 50 parts of ethanol (95% w/w)

5.2.2 Identification test

I.R. spectroscopic studies

Infra red (I.R.) study was carried out on FTIR 840 Shimadzu, Japan equipped with DRS 800. Potassium bromide was used for preparing the sample for I.R. spectroscopic study. The spectrum was recorded using the software IR solution Ver.1.21, in the scanning range of 400-2000 nm, and resolution of 2 cm⁻¹ (Fig. 11) and the peaks are reported in Table 6.

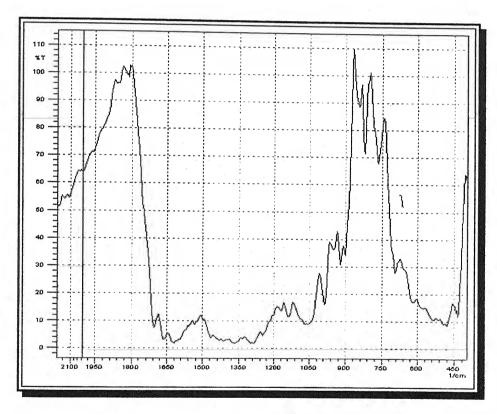


Figure 11 - I.R. spectrum of dexamethasone sample

Table 6 - Peaks in I.R. spectrum indicating various functional groups of dexamethasone sample

S.No.	Functional group	Peak observed	
1	$\Delta^{1,4}$ - diene-3-one	890	
2	11 β – OH	1045	
3	21 – OH	1090	
4	17α – OH	1130	
5	A – ring	1650	
6	C ₃ carbonyl stretching	1660	

5.2.3 U.V. absorption spectrometric studies

 $E_{1\,cm}^{1\%}$ of dexamethasone in ethanol-95% (0.001% w/v) was determined using the Shimadzu 1700 U.V. spectrophotometer and it was found to have absorption of 396 at a 240 nm (As per I.P. 1996: 380 – 410).

5.2.4 Other tests

Loss on drying: On drying up to constant weight at 100°C loss

in weight was found to be 0.2%.

(As per Int. Ph.: NMT 0.5% of its weight).

Specific optical rotation: $\left[\alpha\right]_D^{20^0C} = +74$ of 10 mg/ml dexamethasone in

dioxin; (As per Int. Ph., $[\alpha]_D^{20^{\circ}C} = +72$ to 80).

Residue on ignition: $0.1 \pm 0.03\%$ w/w

(As per Int. Ph.: NMT 0.5% w/w).

Conclusion

On the basis of the above tests, it could be confirmed that the drug sample of dexamethasone is an authentic one.

5.3 ANALYTICAL METHODOLOGY

5.3.1 U.V. absorption spectra

A 50 μ g/ml solution of dexamethasone was prepared in 20% w/w PEG 400 and phosphate buffer saline (PBS) pH 7.4 solution, and scanned for UV absorption in the range of 200 to 400 nm. The UV absorption spectra (Fig. 12) exhibited a λ_{max} (wavelength of maximum absorbance) at 244 nm.

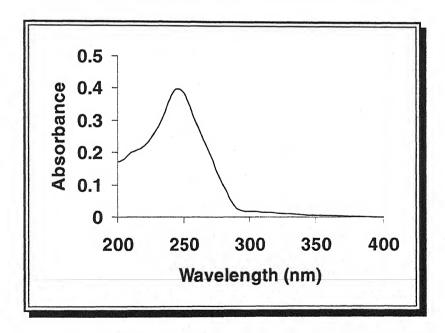


Figure 12 - U.V. absorbtion spectra of dexamethasone

5.3.2 Comparison of UV absorption spectra before and after storage of dexamethasone in 20% w/w PEG 400 and PBS pH 7.4 solution

Drug solution was prepared in 20% w/w PEG 400 and PBS pH 7.4 solution and divided into two portions as under:

- A. Portion scanned U.V. spectrophotometrically immediately after preparation of solution
- B. Portion kept under laboratory conditions and exposed to light for 48 h

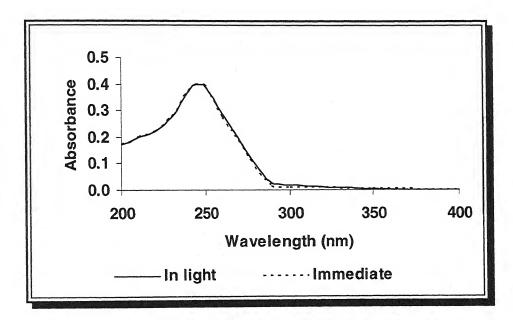


Figure 13 - U.V. absorbance spectra of dexamethasone before and after exposure to light

As shown in the Fig. 13 no change in the λ_{max} was observed when the solution was kept in light. It was therefore not necessary to provide due protection from light.

5.3.3 Calibration curve of dexamethasone

Stock solution of dexamethasone was made by dissolving 50 mg of drug in 500 ml of 20% PEG 400 in PBS pH 7.4. Serial dilutions in the range of 1 to 30 μ g/ml were made from the stock solution and absorbance of these solutions were recorded at the λ_{max} of 244 nm using 20% w/w PEG 400 and PBS pH 7.4 solution as blank (Table 7).

Table 7 - Absorbance values of dexamethasone in 20% w/w PEG 400 and PBS pH 7.4 solution at λ_{max} of 244 nm

S.No.	Concentration of dexamethasone (mcg/ml)	Mean absorbance	± S.D. (n=4)
1	2	0.075	0.003
2	4	0.155	0.004
3	6	0.241	0.013
4	8	0.323	0.012
5	10	0.401	0.029
6	12	0.480	0.027
7	14	0.558	0.027
8	16	0.637	0.020
9	18	0.715	0.042
10	20	0.794	0.038
11	22	0.872	0.025
12	24	0.951	0.022
13	26	1.029	0.052
14	28	1.108	0.058
15	30	1.186	0.075

The absorbance values corresponding to each concentration was then statistically evaluated and plotted as a standard graph with absorbance on y-axis and concentration of dexamethasone (μ g/ml) on x-axis. The calibration curve is shown in Fig. 14. The calibration curve followed Lambert's law in the selected concentration range.

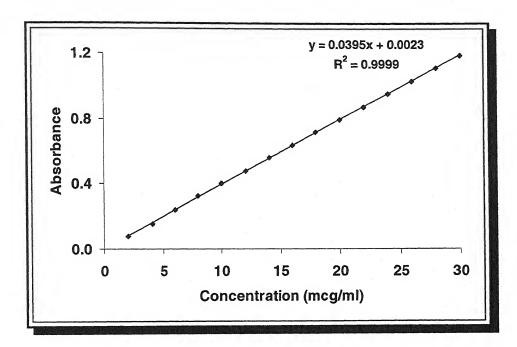


Figure 14 - U.V. absorption spectra of dexamethasone in 20% w/w PEG 400 and PBS pH 7.4 solution at λ_{max} of 244 nm

5.3.4 Method for measurement of dexamethasone in in-vitro drug release studies

One ml sample of receptor medium was withdrawn at defined time intervals. The receptor media was replaced immediately with equal volume of 20% w/w PEG 400 and phosphate buffer saline (PBS) pH 7.4 solution. The sample

withdrawn was filtered through Whatmann filter paper no. 42 and the volume was made up. The samples were analyzed spectrophotometrically at 244 nm. The concentration of drug was read from the calibration curve prepared as reported in section 5.3.3. The cumulative amount of drug permeated was calculated by the following equation:

$$\frac{Drug\ permeated}{(mcg/cm^2)} = \frac{Volume\ of\ receiver\ compartment\ (ml)}{Area\ of\ permeation\ (cm^2)} + \frac{Drug\ lost}{in\ sampling}$$

5.3.5 Analysis of dexamethasone content in the gel formulation

Known quantity of gel was collected into 30 ml capacity screw capped vials with Teflon lined caps and shaken with 10 ml methanol for 2 h to extract out the drug. The alcohol phase was collected and appropriately diluted with 20%w/w PEG 400 and PBS pH 7.4 solution, filtered and the quantity of drug determined UV spectrophotometrically at λ_{max} of 244 nm using the regression equation obtained for the calibration curve as reported in section 5.3.3.

Amount of drug
$$(mg) = \frac{Concentration (mcg / ml) \times Dilution factor}{1000}$$

Percentage drug content and percent recovery is shown in Table 8.

Table 8 - Drug content and percent recovery of dexamethasone from transdermal patches

Formulation	% Drug content	% Recovery	
		Mean	± S.D. (n=4)
AO	99.44	99.44	0.41
LO	99.73	99.73	0.35
NO	99.51	99.51	0.86
00	99.22	99.22	0.63
Control	99.89	99.89	0.12

5.4 PRE-FORMULATION STUDIES FOR PREPARATION OF DEXAMETHASONE FORMULATION

5.4.1 Pseudoternary phase diagram study

Almond oil, olive oil, linseed oil and nutmeg oil were selected as the oil phase. Egg lecithin and IPA were selected as surfactant and cosurfactant, respectively. Distilled water was used as an aqueous phase. Surfactant — cosurfactant mixture (S:CoS) of different weight ratios (1:3, 1:2, 1:1, 1:0, 2:1, 3:1 and 4:1) were chosen. These S:CoS ratios were chosen in both increasing concentration of cosurfactant with respect to surfactant and increasing concentration of surfactant with respect to cosurfactant. Pseudoternary phase diagrams of oil, S:CoS and aqueous phase were developed using the aqueous titration method under moderate magnetic stirring. Slow titration with the aqueous phase was done to each weight ratio of oil and S:CoS. After being

equilibrated, the mixtures were assessed visually and determined as being microemulsions by virtue of their clarity and transparency. The physical state of the microemulsion was marked on a pseudo-three-component phase diagram with the aqueous phase, oil phase and mixture of surfactant and cosurfactant representing the three axis.

5.4.2 Screening of microemulsions

To find out the suitability of oil to be used as the oil phase in microemulsion, the solubility of dexamethasone was determined. Microemulsion were prepared with each oil - S:CoS (almond oil-1:1, olive oil-1:3, linseed oil-3:1 and nutmeg oil-2:1) and water in 1:7:2 ratio. Egg lecithin and isopropyl alcohol (IPA) were selected as surfactant and co surfactant respectively. To it excess amount of dexamethasone was added in 2 ml of each of the selected microemulsion in 5 ml capacity vials, mixed and placed in shaker water bath maintained at $37^{\circ}\text{C} \pm 1.0^{\circ}\text{C}$ for 72 h. The equilibrated samples were removed from the shaker, centrifuged and the supernatant was filtered through Whatman filter no. 4. The concentration of dexamethasone was determined UV spectrophotometrically at the λ_{max} .

5.5 PREPARATION OF MICROEMULSION BASED DEXAMETHASONE FORMULATION

5.5.1 Preparation of microemulsion of dexamethasone

Dexamethasone was added to the mixtures of oil and S:CoS with varying component ratio as described in Table 9. Appropriate amount of water was then added to the mixture drop by drop and the microemulsion containing dexamethasone was obtained by stirring the mixtures at 20 rpm. All microemulsions were stored at $30 \pm 2^{\circ}$ C temperature. Dexamethasone and 0.1% w/w was incorporated in all formulations.

Table 9 - Compositions of the selected microemulsion of dexamethas one (% w/w).

Microemulsion	Formulation codes				
Components (% w/w)	C	AO	00	LO	NO
Dexamethasone	0.1	0.1	0.1	0.1	0.1
Oil	-	10	10	10	10
Egg lecithin		35	17.5	52.5	46.6
IPA	-	35	52.5	17.5	23.3
Distilled water q.s.	100	100	100	100	100
Vehicle (Oil)	Distilled water	Almond oil	Olive oil	Linseed oil	Nutmeg oil

5.5.2 Preparation of microemulsion based dexamethasone gel preparations

Carbopol was selected as the polymer to form gel matrix for microemulsion based hydrogel formulation. Carbopol was slowly mixed with microemulsion under stirring. After carbopol had swelled it was kept overnight to obtain microemulsion based hydrogel.

The control formulation was prepared by adding 0.1% w/w dexamethasone to phosphate buffer saline pH 7.4 and was gelled by addition of carbopol (1%w/w) and triethanolamine.

5.5.3 Fabrication of reservoir type patch of microemulsion gel of dexamethasone

Transdermal patches (reservoir type) of dexamethasone were fabricated by filling microemulsion based dexamethasone gel preparations within a shallow compartment made of drug impermeable backing membrane and a hollow ring shaped device and a drug impermeable backing membrane (laminated aluminum foil). A micro porous adhesive tape of a larger area was stuck onto the impermeable backing membrane to bring the transdermal patch in intimate contact with the skin. The device was closed by a release liner on the open side (Fig. 15).

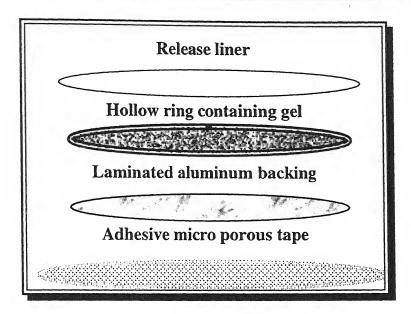


Figure 15 - Design of transdermal patch of microemulsion based dexamethasone gel formulation

The reservoir type transdermal delivery system of dexamethasone comprised of four basic components:

- a. An adhesive backing to bring the patch in intimate contact with the skin and help the patch adhere to the skin.
- b. Drug impermeable backing membrane to avoid loss of solvent and/or formulation
- c. Drug reservoir viscous gel preparation consisting of the therapeutic agent gel preparation
- d. Release liner which protects the integrity of the dosage form and is removed before the use of the transdermal system.

Adhesive backing

The adhesive backing consisted of micro porous adhesive tape commercially available by 3M, which was larger in area then the impermeable backing membrane.

Impermeable backing membrane

dusted off with soft cloth.

It consisted of a laminated aluminum foil available from local vendors and widely used in packing food containers.

5.5.4 Method of application of the transdermal system

The release liner was safely removed and the patch was securely placed over the hairless dorsal abdominal skin. The patch was brought in intimate contact with the skin by cautiously pressing the micro porous adhesive tape to the skin.

5.5.5 Preparation and method of application of abrasive gel

Distilled water was heated to 80°C and adding to it crushed apricot seed (*Prunus armeniaca*). The polymer (hydroxypropyl methylcellulose) 2% w/w was then added slowly and allowed to disperse uniformly. It was then cooled to form viscous gel into which was entrapped crushed apricot shell pieces.

One gram of abrasivegel was topically applied with the help of index finger on the depilated site of application of formulation and was gently massaged onto the skin for a period of two minutes. The crushed apricot pieces were

5.6 CHARACTERIZATION OF DEXAMETHASONE LOADED MICROEMULSION PREPARATION

5.6.1 Droplet size determination

The droplet size distribution and average droplet size of microemulsion were measured using Zetasizer Nano ZS (Malvern Instruments, UK) using water as dispersant at 25°C.

5.6.2 Viscosity measurements of microemulsion based hydrogel

The viscosity measurements were performed at $25^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ using Brookfield viscometer DV-II+ Pro (Middleboro, MA, USA) (Spindle: T-bar and torque >30%).

5.6.3 Stability studies

To overcome the problem of metastable formulation, thermodynamic stability tests were performed. The selected formulations were centrifuged at 3000 rpm for 15 minutes. The formulations that did not show any phase separations were subjected to thermal cycling. Six cycles between refrigeration temperature (4°C) and 45°C with storage at each temperature of not less than 48 h were carried out. The formulations, which were stable at these temperatures, were subjected to a freeze-thaw cycle test. Three freeze-thaw cycles were carried out for the formulation between -21°C and 25°C. The

stability of the microemulsion based hydrogel containing dexamethasone was studied via clarity.

5.6.4 pH measurements

The observed pH values of the samples were measured by a pH meter (Using combined glass electrode) at 20° C \pm 2° C.

5.7 IN-VITRO SKIN PERMEATION STUDIES OF MICROEMULSION BASED DEXAMETHASONE GEL PREPARATIONS

5.7.1 Preparation of rat skin

Albino rats were used in the present study. The rats that had been sacrificed after other experimental work were used in the study; care was taken that only such animals be used whose skin was not affected in the experimental procedure. The dorsal skin of the rat was shaved with the help of hair clipper and full thickness skin was surgically removed. The subcutaneous fat of the skin was removed with a pair of scissors. The cleaned skin was wrapped in aluminum foil and stored in freezer at –21°C until further use. The skin was slowly brought to room temperature before using the same.

5.7.2 Fabrication of apparatus for in-vitro skin permeation studies

In-vitro skin permeation studies were carried out using modified Keshary Chien cell. The diffusion cells were fabricated locally by Rama Scientific Industries, Govindpuri, New Delhi. (Fig. 16). The material used for fabrication was type I borosilicate glass.

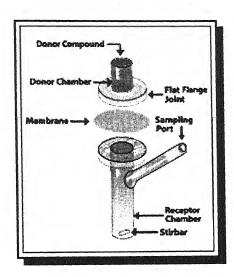


Figure 16 - Design of Keshary Chien diffusion cell used in *in-vitro* skin permeation studies

The assembly consisted of two chambers: a. the open upper chamber known as donor chamber and, b. the lower receptor chamber containing a sampling port and Teflon coated magnetic bead. The internal diameter of the recipient compartment was also 0.8 cm. The capacity of the receiver chamber was 11 ml the area of diffusion was 1.0 cm².

There were hooks on both upper and lower chamber for attachment of spring so that the two chambers could be securely attached. The receptor chamber was maintained at a temperature of 37 ± 2 °C.

5.7.3 In vitro skin permeation studies of dexamethasone across rat skin

In vitro skin permeation studies were performed on a Keshary Chien diffusion cell. The full-thickness rat skin was used for the permeation experiments. The skin was brought to room temperature and mounted between the donor and receiver compartment of the Keshary Chien diffusion cell, the stratum corneum side faced the donor compartment and the dermal side faced the receiver compartment. The skin was clamped between the donor and the receptor chamber of Keshary Chien diffusion cell with an effective diffusion area of 1.0 cm² and 11 ml receptor cell volume. The receptor chamber was filled with freshly prepared 20% PEG 400 in phosphate buffer saline (pH 7.4). The diffusion cell was maintained at 37°C ± 2°C and the solution in the receptor chambers was stirred continuously with a magnetic bead. Receptor fluid was replaced every 30 minutes until the skin was equilibrated. The skin was found to completely stabilize in a maximum of 6 h and the receptor fluid showed negligible absorbance indicating complete stabilization of the skin. After complete stabilization of the skin, 2.0 g of microemulsion based dexamethasone gel was placed in the donor chamber. In case of pretreatment with abrading gel the skin was clamped and to it was applied 1 g of abrading gel and was massaged onto the skin for 2 min. The crushed apricot seed debris were dusted off with a soft cloth, the donor chamber was mounted and the experiment carried as outlined. The sampling port was also covered with Parafilm M® to prevent evaporative loss of receptor medium. At 2, 4, 6, 8, 10, 12, 20, 22, and 24 h, 2 ml of the solution in the acceptor chamber was removed and analyzed for drug content by UV spectrophotometer at λ_{max} of 244 nm using 20% w/w PEG 400 and PBS pH 7.4 solution as blank as mentioned in section 5.3.4. The receptor volume was immediately made up with an equal volume of receptor fluid. All experiments were performed in quadruplicate. The microemulsion based dexamethasone gel formulations were designated as C, AO, OO, LO and NO for formulations prepared from distilled water (control), almond oil, olive oil, linseed oil and nutmeg oil respectively and when applied after pretreatment with abrasive gel were designated by adding a suffix "A" to the formulation code, as CA, AOA, OOA, LOA and NOA for formulations prepared from distilled water (control), almond oil, olive oil, linseed oil and nutmeg oil were designated as respectively.

5.8 PHARMACOLOGICAL STUDIES

5.8.1 Animal studies

The reported experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), constituted under the provision of Section 15 of the Prevention of Cruelty to Animals Act, 1960 of the Indian constitution. Experiments were approved by the Institutional Animal Ethics Committee of Bundelkhand University (registration no. 716/02/a/CPCSEA) ref. no. I4/2007.

5.8.2 Skin irritation study

Various preparations, when applied dermally, might elicit skin irritation. Therefore to assess the skin sensitizing potential, dexamethasone patch was applied onto the dorsal skin of albino Wistar rats. The animals were housed in polypropylene cages, with free access to standard laboratory diet and water. Animals were acclimatized for at least seven days before experimentation. The dorsal abdominal skin of the rats was shaved 24 h prior to study. The formulations were applied and the site of application was occluded with gauze and covered with non-sensitizing micro-porous tape. Erythema values for formulations with and without pretreatment with abrading gel were recorded. After 24 h, the patch was removed and the score of erythema was determined by Drazie test 175 as follows: 0 - no erythema; 1 - very slight erythema (barely

perceptible); 2 - well-defined erythema; 3 - moderate to severe erythema and 4 - severe erythema (beet redness) to slight eschar formation (injuries in depth).

5.8.3 In vivo anti-inflammatory studies

Wistar albino rats (220–250g) were assigned to weight-balanced groups (n = 4). The experimental groups received the different formulations, while the control group was treated with placebo only. 2 g of the different formulations were spread over 9 cm² of dorsal skin after removing the hair with a clipper. After 2 h, 0.05 ml of a 0.5% carrageenan suspension was injected into the sub- plantar area of the left hind paw. The activity was measured by measuring the changes in paw volumes with a screw gauge (Mitutoyo, Kanagawa, Japan) 4 h after carrageenan injection. The right hind paw served as control was treated with physiological saline solution without carrageenan ²⁸. Four hour after the carrageenan injection the degree of paw swelling was calculated as:

Swelling (%)=
$$\frac{Vt-V}{V}X100$$

where, V_t is the volume of the carrageenan-treated paw, V is that of the non-treated paw.

$$Inibition(\%) = \frac{Sc - St}{Sc} X 100$$

where, Sc is the swelling of the control paw, St is that of the test formulation treated paw.

5.9 DATA ANALYSIS

5.9.1 Data analysis of in-vitro permeation studies

The *in-vitro* skin flux was determined from Fick's law of diffusion considering the transport of drugs across the skin barrier as a process of passive diffusion.

Various parameters were obtained using the equations reported below 176:

$$J_{SS} = \frac{(dM)}{dt} \cdot \frac{1}{A} = Kp \times \Delta C$$

and

$$K_p = \frac{J_{SS}}{C_o}$$

 J_{SS} is the skin flux ($\mu g/cm^2/h$), dM/dt is the amount of drug permeated per unit of time, A is the permeation area (cm^2), Kp is the permeability coefficient (cm h^{-1}), ΔC is the concentration gradient and C_0 is the donor phase concentration. In all the experiments, the concentrations in the donor cell remained constant, the receiver cell concentrations did not exceed 10% of the donor cell concentrations, and ΔC was assumed to be equal to the donor cell concentrations.

The steady-state flux J_{SS} was determined from the slope of the linear portion of the cumulative amount permeated per unit area versus time plot. The lag time (T $_{lag}$) was determined by extrapolating the linear portion of the curve to the abscissa.

The Kp can also be determined as:

$$K_p = \frac{D x P}{h}$$

D is the diffusion coefficient of the drug into the stratum corneum which reflects the facility for the molecules to move through the membrane strata and is a function of the molecular structure of the diffusant. P is the partition coefficient of drug between stratum corneum and vehicle of formulation. h is the barrier thickness for skin (stratum corneum thickness, 15.4 μ m), assuming that the SC represents the main rate-limiting barrier. Partition coefficient was calculated from values of Kp and D while D was approximated indirectly from the lag time $(T_{lag})^{177}$

$$D = \frac{h^2}{6T_{lag}}$$

Enhancement ratio (ER) was calculated from the following equation:

Enhancement ratio (ER) =
$$\frac{J_{ss} \text{ of test formulation}}{J_{ss} \text{ of control formulation}}$$

5.9.2 Statistical analysis

All skin permeation experiments were repeated four times and data were expressed as the mean value±S.D. The results were analyzed by Student's t-test using Statistica for windows (version 5.0) from StatSoft, Inc., USA. The results were evaluated at probability level of 0.05.

EXPERIMENTAL

PRONIOSOMAL GEL BASED

TRANSDERMAL

DRUG DELIVERY SYSTEM OF BIROXICAM

6. EXPERIMENTAL – PRONIOSOMAL GEL BASED TRANSDERMAL DRUG DELIVERY SYSTEM OF PIROXICAM

6.1 MATERIALS

S.No.	Name of Ingredient	Source		
1	Piroxicam	Torrent Pharmaceuticals, Ahemdabad, India.		
2	Span 20, span 40, span 60, span 80, chloroform, isopropyl alcohol (IPA), maltodextrin, sorbitol, cholesterol, Carbopol 934	Central Drug House, New Delhi, India.		
3	Egg lecithin	Himedia, Mumbai, India.		

6.2 CHARACTERIZATION OF PIROXICAM

6.2.1 Physical characteristics of the drug

Nature

: Powdered solid

Colour

: Off white

Odour

: Odourless

Taste

: Bitter

Melting point

: 198 ± 1°C

6.2.2 Identification test

I.R. spectroscopic studies

Infra red (I.R.) study was carried out on FTIR 840 Shimadzu, Japan equipped with DRS 800. Potassium bromide was used for preparing the sample for I.R. spectroscopic study. The spectrum (Fig. 17) was recorded using the software IRsolution Ver.1.21, in the scanning range of 400-2000 nm and resolution of 2 cm⁻¹. Peaks corresponding to various functional groups were reported in Table 10.

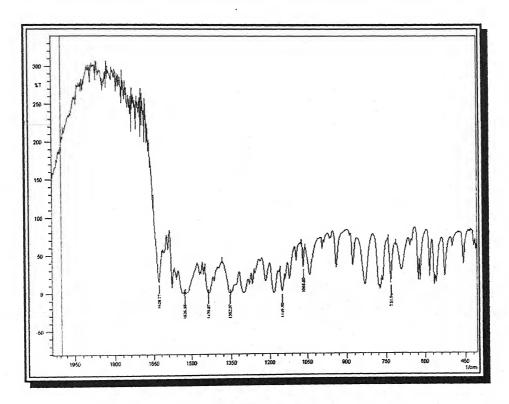


Figure 17 - I.R. spectrum of piroxicam sample

Table 10 - Peaks in I.R. spectrum indicating various functional groups of piroxicam sample

prioricam sample						
S.No.	Functional group	Peak observed				
1	Ortho di-substituted phenyl stretching	731.94				
2	Stretching of –SO ₂ N group	1066.50				
3	Stretching of symmetric methyl group	1352.97				
4	Stretching of asymmetric methyl group	1436.87				
5	Stretching of second amide bond	1536.55				
6	Stretching of carbonyl amide bond	1628.77				
7	Over tone band	1800-2000				

Conclusion

On the basis of the above tests, it could be confirmed that the drug sample of piroxicam is an authentic one.

6.3 ANALYTICAL METHODOLOGY

6.3.1 UV absorption spectrometric studies

A 100 μ g/ml solution of piroxicam was prepared in 30% w/w PEG 400 in PBS of pH 7.4 and scanned for UV absorption in the range of 200 to 400 nm. The UV absorption spectra (Fig. 18) exhibited a λ_{max} (wavelength of maximum absorbance) at 356 nm.

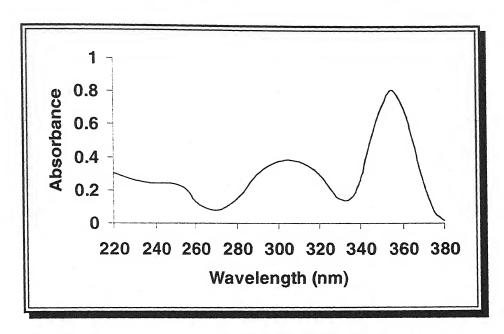


Figure 18 - U.V. absorption spectra of piroxicam in 30% w/w PEG 400 PBS of pH 7.4

6.3.2 Comparison of UV absorption spectra before and after storage of piroxicam in 30% PEG 400 in PBS pH 7.4

Drug solution was prepared in 30% w/w PEG 400 and PBS pH 7.4 solution and divided into two portions (A and B) as under:

- A. Portion scanned U.V. spectrophotometrically immediately after preparation of solution
- B. Portion kept under laboratory conditions and exposed to light for 48 h

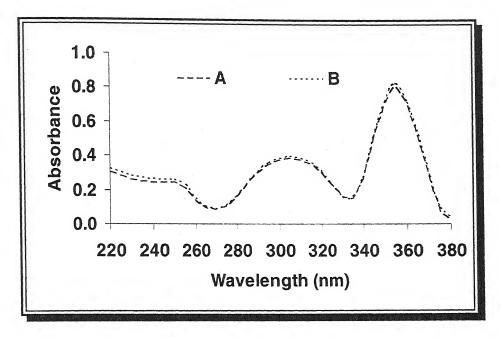


Figure 19 - Absorbance spectra of dexamethasone before and after exposure to light

- A- Spectra of sample scanned immediately
- B- Spectra of sample scanned after 48 h

As shown in the Fig. 19 no change in the λ_{max} was observed when the solution was kept in light. It was therefore not necessary to perform all experiments in dark or due protection from light be provided.

6.3.3 Calibration curve of piroxicam

Stock solution of piroxicam was prepared by dissolving 100mg of drug in 100 ml of 30% w/w PEG 400 in PBS pH 7.4. Serial dilutions in the range of 1 to 35 μ g/ml were made from the stock solution and absorbance of these solutions were recorded at the λ_{max} of 356 nm using 30% w/w PEG 400 and PBS pH 7.4 solution (Table 11).

Table 11 - Absorbance values of piroxicam in 30% w/w PEG 400 in PBS pH 7.4 at λ_{max} of 356 nm

S.No.	Concentration of piroxicam (mcg/ml)	Mean absorbance	± S.D. (n=4)
1	2	0.085	0.001
2	5	0.157	0.001
3	10	0.316	0.005
4	15	0.475	0.007
5	20	0.634	0.012
6	25	0.798	0.013
7	30	0.962	0.014
8	35	1.111	0.011

The absorbance values corresponding to each concentration were then statistically evaluated and plotted as a standard graph, between absorbance on y-axis and concentration of piroxicam ($\mu g/ml$) on x-axis. The calibration curve is shown in Fig. 20. The calibration curve followed Lambert's law in the selected concentration range.

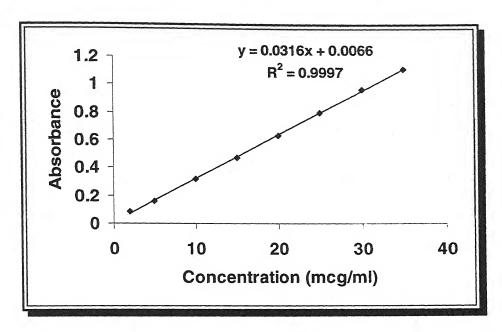


Figure 20 - Calibration curve of piroxicam in 30% w/w PEG 400 in PBS of pH 7.4 at λ_{max} of 356 nm

6.3.4 Method for measurement of piroxicam in *in-vitro* drug release studies

Sample (2 ml) of receptor medium was withdrawn at defined time intervals. The receptor media was replaced immediately with equal volume of fresh 30% w/w PEG 400 in PBS of pH 7.4. The sample withdrawn was filtered through Whatmann filter paper no. 42 and the volume was made up. The samples were analyzed spectrophotometrically at 356 nm. The concentration of drug was read from the calibration curve prepared as reported in section 6.3.3. The cumulative amount of drug permeated was calculated by the following equation:

$$\frac{Drug \ permeated}{(mcg/cm^2)} = \frac{\begin{array}{c} Concentration(mcg/ml)*Dilution \ factor* \\ \hline Volume \ of \ receiver \ compartment(ml) \\ \hline Area \ of \ permeation(cm^2) \\ \end{array}}{\begin{array}{c} + \ Drug \ lost \\ in \ sampling \\ \end{array}}$$

6.3.5 Analysis of drug content in the piroxicam gel formulation

Gel was collected into 30 ml capacity screw capped vials with Teflon lined caps and shaken with ethanol to extract out the drug. The drug present in ethanol was determined after diluting with 30% w/w PEG 400 PBS of pH 7.4 UV spectrophotometrically at λ_{max} of 356 nm using the regression equation obtained for the calibration curve as reported in section 6.3.3.

Amount of drug
$$(mg) = \frac{Concentration (mcg / ml) \times Dilution factor}{1000}$$

Percentage drug content and percent recovery is shown in Table 12.

Table 12 - Drug content and percent recovery of piroxicam from transdermal patches

Formulation	% Drug content	% Recovery		
Tormulation	% Drug content	Mean	± S.D. (n=4)	
S6L	99.26	99.26	0.68	
S6LM	98.48	98.48	0.83	
S6LS	98.78	98.78	0.79	
Control	99.49	99.49	0.24	

6.4 PREPARATION OF PIROXICAM FORMULATION

Various proniosomal preparations were formulated using surfactant, cholesterol, lecithin and Piroxicam. The compositions of different proniosomal formulations prepared are listed in Table 13.

6.4.1 Conventional proniosome

Proniosomes were prepared using the reported method (Perrett et al., 1991)²⁸. Appropriate amounts of surfactant (Span), cholesterol, lecithin, and piroxicam in isopropyl alcohol were taken in a wide-mouth glass vial. The open end of the glass vial was covered and the tube was warmed in a water bath at 65 °C till the surfactant mixture dissolved completely. The preparation on congealing formed proniosome. The preparation obtained from span 20, 40, 60 and 80 were designated as S2, S4, S6 and S8 respectively while the preparation obtained from span 60 containing lecithin was designated as S6L.

6.4.2 Maltodextrin based proniosome

Appropriate amounts of surfactant (Span 60), cholesterol, lecithin and piroxicam were dissolved in chloroform and isopropyl alcohol mixture (4:1) and were added to a 100 ml round bottom flask containing 0.5 g of maltodextrin powder. The flask was attached to the rotary evaporator maintained at a temperature of 65°C using water bath and the flask was rotated at 60 rpm under vacuum until the powder appeared to be dry and free

flowing. The dried material was removed from the evaporator and kept under vacuum overnight. The preparation obtained was designated as S6LM.

6.4.3 Sorbitol based proniosomes

Sorbitol (0.5 g) was placed in a 100 ml round bottom flask attached to a rotary evaporator. Surfactant, cholesterol, lecithin and piroxicam were mixed in chloroform and isopropyl alcohol mixture (4:1) was added slowly on to sorbitol powder bed. Care was taken not to over wet the powder base. The rotary evaporator was maintained at a temperature of 65°C using water bath and the flask was rotated at 60 rpm under vacuum so as to dry the powder base before further addition of surfactant mixture. The dried material was finally removed and kept under vacuum overnight. The preparation obtained was designated as S6LS.

6.4.4 Preparation of piroxicam niosomal gel

Proniosome powder was weighed into screw cap vials to which was added water at 80°C. The vials were vortex mixed for complete and uniform hydration. The niosomal preparations were then converted into gel by adding Carbopol 934 (1%w/w). The final drug concentration achieved was 0.5%w/w.

Table 13 - Composition of proniosomal formulation of piroxicam

Proniosomal formulation code	Span 20 (mg)	Span 40 (mg)	Span 60 (mg)	Span 80 (mg)	Cholesterol (mg)	Lecithin (mg)
S2	360	-	-	**	40	-
S4	-	360	-	-	40	-
S6	-	-	360	_	40	-
S8	-	-	-	360	40	-
S6L	-	_	180	_	40	180
S6LM	-	-	180	-	40	180
S6LS	-	-	180	-	40	180

6.4.5 Preparation of piroxicam carbopol gel

0.5% w/w piroxicam was dissolved/suspended in saline phosphate buffer pH 7.4 and to it was added carbopol 934 (1% w/w). The gel was finally obtained by addition of triethanolamine.

6.4.6 Fabrication of patch of piroxicam gel system

Transdermal patches (reservoir type) of piroxicam were fabricated by filling piroxicam gel preparation (1g/cm²) within a shallow compartment made of drug impermeable backing membrane and a hollow ring shaped device and a drug impermeable backing membrane (laminated aluminum foil). A micro porous adhesive tape of a larger area was stuck onto the impermeable backing

membrane to bring the transdermal patch in intimate contact with the skin.

The device was closed by a release liner on the open side (Fig. 21).

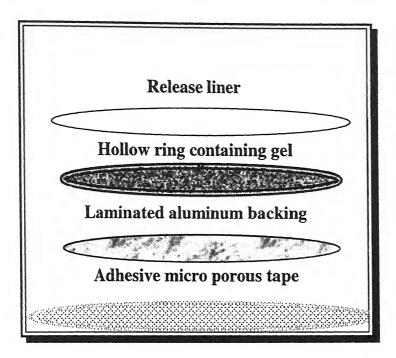


Figure 21 - Design of transdermal patch of piroxicam

The reservoir type transdermal delivery system of piroxicam comprised of four basic components:

- a. An adhesive backing to bring the patch in intimate contact with the skin and help the patch adhere to the skin.
- b. Drug impermeable backing membrane to avoid loss of solvent and/or formulation.
- c. Drug reservoir viscous gel preparation consisting of the therapeutic agent.

d. Release liner which protects the integrity of the dosage form and is removed before the use of the transdermal system.

Adhesive backing

The adhesive backing consisted of micro porous adhesive tape commercially available by 3M, which was larger in area then the impermeable backing membrane.

Impermeable backing membrane

It consisted of a laminated aluminum foil available from local vendors and widely used in packing food containers.

6.4.7 Method of application of the transdermal system

The release liner was safely removed and the patch was securely placed over the hairless dorsal abdominal skin. The patch was brought in intimate contact with the skin by cautiously pressing the micro porous adhesive tape to the skin.

6.5 EVALUATION

6.5.1 Encapsulation efficiency

Weighed quantity of proniosomes (S2, S4, S6 and S8) were hydrated with saline and placed in a glass tube to which a Hi Media dialysis membrane was securely attached and dialyzed into 600 ml of saline (0.9%w/v) for 8h with

three washings. The niosomes were collected and lysed using ethanol and suspended in 30% PEG 400 – phosphate buffer saline pH 7.4. The resulting solution was filtered and analyzed spectrophotometrically at 356 nm.

Encapsulation efficiency (%EE) was calculated by the following equation:

%EE =
$$\frac{\text{Ct} - \text{Cf}}{\text{Ct}} \times 100$$

where, Ct is the total concentration of drug and Cf is the concentration of free drug.

6.5.2 Polarized microscopy and vesicle size analysis

A drop of hydrated proniososomal formulation was placed in a cavity slide and observed under microscope with polarized light. The niosomes were visualized by polaroid microscope (Nikon HFX-DX, Labophot Microscope, Germany). Photomicrographs were taken at suitable magnifications. The vesicle size of 300 niosomes was recorded with optical micrometer.

6.5.3 Scanning electron microscopy (SEM)

Proniosome powders were affixed to double-sided carbon tape, positioned on an aluminum stub, and excess powder removed. The stubs were stored under vacuum overnight. The samples were sputter-coated with gold. Electron micrographs were obtained using scanning electron microscope operating at 15 kV accelerating voltage (LEO 435 VP Electron Microscopy Ltd, UK).

6.6 IN-VITRO SKIN PERMEATION STUDIES OF PIROXICAM GEL PREPARATIONS

6.6.1 Preparation of rat skin

Albino rats were used in the present study. The rats that had been sacrificed after other experimental work were used in the study; care was taken that only such animals be used whose skin was not affected in the experimental procedure. The dorsal skin of the rat was shaved with the help of hair clipper and full thickness skin was surgically removed. The subcutaneous fat of the skin was removed with a pair of scissors. The cleaned skin was wrapped in aluminum foil and stored in freezer at -21°C until further use. The skin was slowly brought to room temperature before using the same.

6.6.2 Fabrication of apparatus for in-vitro skin permeation studies

In-vitro skin permeation studies were carried out using modified Keshary Chien cell. The diffusion cells were fabricated locally by Rama Scientific Industries, Govindpuri, New Delhi (Fig. 22). The material used for fabrication was type I borosilicate glass.

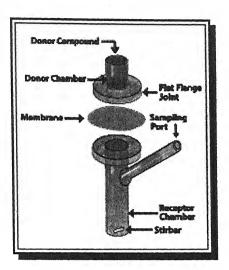


Figure 22 - Design of Keshary Chien diffusion cell used in *in-vitro* skin permeation studies

The assembly consisted of two chambers: a. the open upper chamber known as donor chamber and, b. the lower receptor chamber containing a sampling port and contained a Teflon coated magnetic bead. The capacity of the receiver chamber was 11 ml and the area of diffusion was 2.0 cm².

There were hooks on both upper and lower chamber for attachment of spring so that the two chambers could be securely attached. The receptor chamber was maintained at a temperature of $37 \pm 1^{\circ}$ C.

6.6.3 In-vitro permeation studies of piroxicam across rat skin

In-vitro skin permeation studies were performed on a Keshary Chien diffusion cell with an effective diffusional area of 2.0 cm² and 11 ml of receiver chamber capacity using rat abdominal skin. The skin was brought to room

temperature and mounted between the donor and receiver compartment of the Keshary Chien diffusion cell, the stratum corneum side faced the donor compartment and the dermal side faced the receiver compartment. Initially the donor compartment was empty and the receiver chamber was filled with 30% w/w PEG 400 in PBS of pH 7.4. The receiver fluid was stirred with a magnetic bead and the temperature was maintained at $37 \pm 1^{\circ}$ C. The PBS was replaced every 30 minutes until the skin equilibrated. The skin was found to completely stabilize in a maximum of 6 h and the receptor fluid showed negligible absorbance indicating complete stabilization of the skin.

After complete stabilization of the skin, 2 g of gel was placed into donor compartment and sealed with Parafilm M^{\circledast} to provide occlusive conditions. Samples were withdrawn at regular intervals (2, 4, 6, 8, 10, 12, 18, 22, and 24 h) and analyzed for drug content by UV spectrophotometer at λ_{max} of 356 nm using 30% w/w PEG 400 PBS of pH 7.4 as blank as mentioned in section 6.3.4. The receptor volume was immediately replaced with fresh receptor medium. The sampling port was also covered with Parafilm M^{\circledast} to prevent evaporative loss of receptor medium.

6.7 PHARMACOLOGICAL STUDIES

6.7.1 Animal studies

The experimental protocol was approved by the institutional animal ethical committee, Institute of Pharmacy, Bundelkhand University, Jhansi. The reported experiments were carried out in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), constituted under the provision of Section 15 of the Prevention of Cruelty to Animals Act, 1960 of the Indian constitution. Experiments were approved by the Institutional Animal Ethics Committee of Bundelkhand University (Registration no. 716/02/a/CPCSEA).

6.7.2 Skin irritation study

To assess the skin sensitizing potential piroxicam transdermal patch was applied onto the dorsal skin of rats (220–250 g) of either sex. The animals were housed in polypropylene cages, with free access to standard laboratory diet and water. They animals were acclimatized for at least seven days before experimentation. The dorsal abdominal skin of the rats was shaved 24 h prior to study and abraded before gel application. The site of application was occluded with gauze and covered with non-sensitizing micro-porous tape. After 24 h, the gel was removed and the score of erythema was determined by Drazie test 175 as follows: 0 - no erythema; 1 - very slight erythema (barely

perceptible); 2 - well-defined erythema; 3 - moderate to severe erythema. and 4 - severe erythema (beet redness) to slight eschar formation (injuries in depth).

6.7.3 Carrageenan induced paw edema

Wistar rats (220–250 g) were assigned to weight-balanced groups (n = 6). The experimental groups received different formulations, while the control group was treated with placebo only. In the experiment, 2 g of the different formulations (S6L, S6LM and S6LS) were applied over 9 cm² as transdermal patch on the dorsal skin after removing the hair with a clipper. After 2 h, 0.05ml of a 0.5% carrageenan suspension was injected into the subplantar area of the left hind paw. The activity was measured by measuring the changes in paw volumes with a screw gauge (Mitutoyo, Kanagawa, Japan) 4 h after carrageenan injection. The right hind paw served as control was treated with physiological saline solution. The degree of paw swelling and inhibition in inflammation was calculated as:

Swelling (%)=
$$\frac{Vt-V}{V}X100$$

where, V_t is the volume of the carrageenan-treated paw, V is that of the non-treated paw.

$$Inibition(\%) = \frac{Sc - St}{Sc} X 100$$

where, Sc is the swelling of the control paw, St is that of the test formulation treated paw. The AUC (area under the curve) was determined by trapezoidal method.

6.8 DATA ANALYSIS

6.8.1 Calculation for predicting desired flux (K_0) required to be achieved for attaining effective plasma concentration

Using the equation:

$$AK_0 = C_p V_d K_{el}$$

The desired flux required to achieve the effective plasma concentration can be calculated

from:

 C_p , the therapeutic plasma concentration (ng/ml); = 2000 ng/ml

V_d, the volume of distribution: 9.8 L

 K_{el} , the elimination rate from plasma: 0.017 h^{-1}

and assuming that area of applicable system: 25 cm²

the target permeation rate (K_0) of the drug was found to be: 13.10 μ g/cm²/hr. In order to achieve the desired C_p the formulation must achieve the calculated flux on application of a patch of 25 cm². However, a patch of larger

(maximum 50 cm²) or smaller area can be employed.

6.8.2 Data analysis of in-vitro permeation studies

The *in-vitro* skin flux was determined from Fick's law of diffusion considering the transport of drugs across the skin barrier as a process of passive diffusion.

Various parameters were obtained using the equations reported below 176:

$$J_{SS} = \frac{(dM)}{dt} \cdot \frac{1}{A} = Kp \times \Delta C$$

and

$$K_p = \frac{J_{SS}}{C_o}$$

 J_{SS} is the skin flux (μ g/cm²/h), dM/dt is the amount of drug permeated per unit of time, A is the permeation area (cm²), Kp is the permeability coefficient (cm h¹), Δ C is the concentration gradient and C_0 is the donor phase concentration. In all the experiments, the concentrations in the donor cell remained constant, the receiver cell concentrations did not exceed 10% of the donor cell concentrations, and Δ C was assumed to be equal to the donor cell concentrations.

The steady-state flux J_{SS} was determined from the slope of the linear portion of the cumulative amount permeated per unit area versus time plot. The lag time (T $_{lag}$) was determined by extrapolating the linear portion of the curve to the abscissa.

The Kp can also be determined as:

$$K_p = \frac{D x P}{h}$$

D is the diffusion coefficient of the drug into the stratum corneum which reflects the facility for the molecules to move through the membrane strata and is a function of the molecular structure of the diffusant. P is the partition coefficient of drug between stratum corneum and vehicle of formulation. h is the barrier thickness for skin (stratum corneum thickness, 15.4 μ m), assuming that the SC represents the main rate-limiting barrier. Partition coefficient was calculated from values of Kp and D while D was approximated indirectly from the lag time $(T_{lag})^{177}$

$$D = \frac{h^2}{6T_{lag}}$$

Enhancement ratio (ER) was calculated from the following equation:

Enhancement ratio (ER) =
$$\frac{J_{SS} \text{ of test formulation}}{J_{SS} \text{ of control formulation}}$$

6.8.3 Statistical analysis

The data obtained was expressed as the mean value \pm S.D. The results were analyzed by Student's t-test using Statistica for windows (version 5.0) from StatSoft, Inc., USA. The results were evaluated at probability level of 0.05.

RESULTS AND DISCUSSION

HYDROGEL BASED TRANSDERMAL SYSTEM OF KETOROLAC

- 7. RESULTS AND DISCUSSION HYDROGEL

 BASED TRANSDERMAL SYSTEM OF

 KETOROLAC
- 7.1 IN-VITRO SKIN PERMEATION STUDIES OF KETOROLAC GEL PREPARATIONS
- 7.1.1 Effect of pH on *in-vitro* skin permeation of ketorolac through rat skin

The effect of pH on gel containing ketorolac (2% w/w) and HPMC (2% w/w) on permeation rate of ketorolac was studied at pH 5.4, 6.4 and 7.4. From the results it was concluded that higher permeation of ketorolac occurred at lower pH (Fig. 23). Flux attained for gel system prepared in pH 5.4, 6.4 and 7.4 were 1.24. 0.99 and 0.79 µg/cm²/h and observed T_{lag} was 7.70, 8.99 and 9.60h respectively. The permeation of a molecule depends primarily on its solubility and its state (ionized/unionized). Since the drug was present at sub saturation concentration and was completely soluble at all pH it was probably the degree of unionized to ionized fraction that affected permeation rate across skin. Thus PBS solution pH 5.4 was selected for further studies.

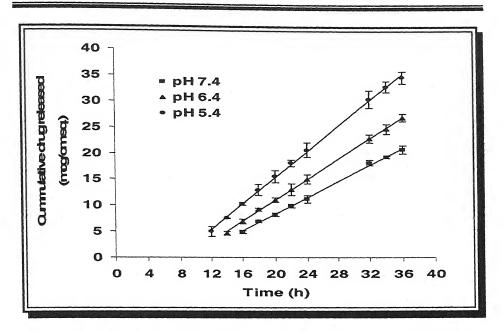


Figure 23 - In-vitro permeation profile of ketorolac through rat abdominal skin from gel system containing ketorolac (2% w/w), HPMC (2% w/w) and formulated using PBS solutions of different pH

7.1.2 Effect of various alcohols on *in-vitro* skin permeation of ketorolac

Various alcohols, viz. ethanol, n-propanol, IPA, n-butanol, n-pentanol and PG at 10%w/w were evaluated for their ability to enhance permeation of ketorolac. Ketorolac gels were prepared in PBS solution pH 5.4. Enhancement ratio was calculated by comparing the steady state flux of ketorolac from alcohol containing gel system and that of the gel system at optimized pH. The release profile is presented in Fig. 24. From the study it was perceived that smaller chain alcohols were more efficient in enhancing the dermal permeation. The shift of hydroxyl group away from the center to the edge side

in the molecular structure considerably reduced the permeation. Highest permeation coefficients were observed for IPA and ethanol. The steady state flux achieved were 4.67 and 3.91 $\mu g/cm^2/h$ respectively but a reduced lag time of 3.26 h was noted for ethanol in comparison to 3.55h for IPA. Other alcohols viz. n-propanol, n-butanol and n-pentanol showed a flux of 3.53, 3.18 and $2.71 \ \mu g/cm^2/h$ respectively. Despite the fact that ethanol demonstrated an enhanced diffusivity, higher Q24 (amount of drug permeated across skin at the end of 24h) was achieved for IPA. Alcohol probably extracts some of the lipid fraction from within the stratum corneum thereby enhancing permeation. Various findings also reveal that longer chain alcohols possess higher enhancement potential towards lipophilic drug¹⁷⁸ and poor enhancement towards hydrophilic drugs. 179 Reports concerning the efficacy of propylene glycol as a permeation enhancer are mixed; evidence suggests only a very mild enhancement effect towards estradiol and 5-fluorouracil. 180 In case of ketorolac a lag time of 5.68 h is observed with propylene glycol as alcohol component of the gel system and the flux attained was 5.68 µg/cm²/h.

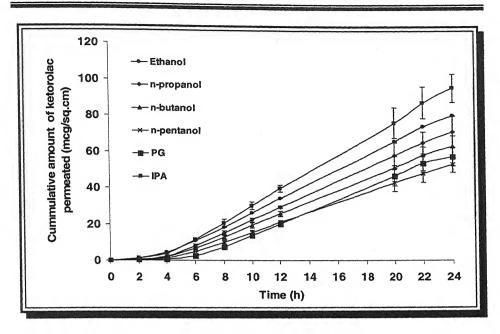


Figure 24 - In-vitro permeation profile of ketorolac through rat abdominal skin from gel system containing ketorolac (2% w/w), HPMC (2% w/w) and different alcohols at 5% w/w concentration formulated using PBS solution of pH 5.4

Increasing the concentration of IPA further enhanced permeation of ketorolac (Fig. 25). An enhancement ratio of 14.55 was attained at 25%w/w IPA concentration. There was an exponential (1.9167e^{0.0895x}, R²=0.9996) rise in ketorolac flux (Fig. 26).

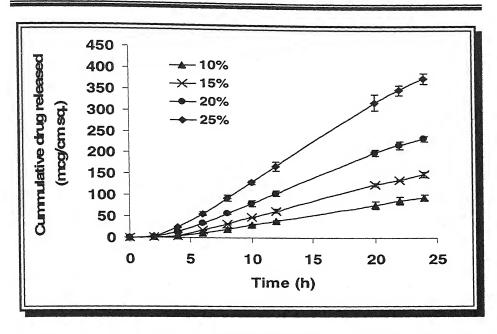


Figure 25 - *In-vitro* permeation profile of ketorolac across rat abdominal skin from gel system containing ketorolac (2%w/w), HPMC (2%w/w) and IPA at varying concentrations formulated in PBS solution of pH 5.4.

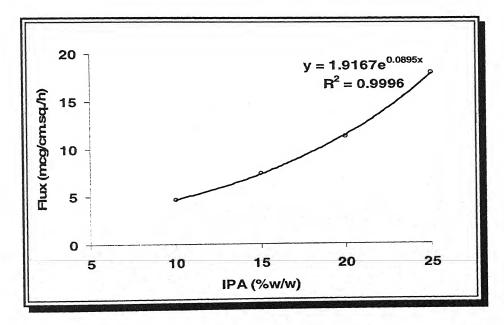


Figure 26 - Flux for ketorolac across rat abdominal skin from gel system containing ketorolac (2% w/w), HPMC (2% w/w) and IPA at varying concentrations formulated in PBS solution of pH 5.4.

7.1.3 Effect of permeation enhancers on *in-vitro* skin permeation of ketorolac

Effect of various chemical permeation enhancers on the permeation of ketorolac from transdermal formulations across rat abdominal skin was investigated. The enhancers evaluated were DMSO, eucalyptus oil, d-limonene and transcutol. The effectiveness of the enhancer was determined by comparing the steady state flux of ketorolac from chemical enhancer containing gel system and that of control gel system without enhancer. *Invitro* permeation profile is presented in Fig. 27. Among the penetration enhancers evaluated the following order was observed; eucalyptus oil> transcutol >DMSO> d-limonene.

DMSO demonstrated a flux of $27.97~\mu g/cm^2/h$ and a T_{lag} of 1.97~h. There was an enhancement of 1.55 times. Being a powerful solvent DMSO can mix isothermally with water, it can displace water from the lipid head groups creating gaps around these head groups. DMSO is also capable of displacing water bound to protein head groups. Moreover due to its solvent power, high levels of sulfoxide within the membrane can improve drug partitioning and thus increase the flux. 181

Transcutol showed a flux of $29.72~\mu\text{g/cm}^2/\text{h}$ and T_{lag} of 2.05~h with enhancement ratio of 1.65. Recent studies have shown that transcutol significantly increases the percutaneous penetration of various active

substances, particularly when used in combination with suitable cosolvents. Particularly when used in combination with suitable cosolvents. Transcutol demonstrated enhanced flux in comparison to DMSO but failed to reduce the lag time. This probably could be due to enhanced accumulation of ketorolac in skin. This enhancer has also shown skin accumulation of topically applied compounds without an increase in transdermal permeation. 187

Essential oils like eucalyptus oil are reported to be effective penetration enhancers for 5-flouorouracil traversing human skin in vivo with maximum enhancement ratio of 34-fold. 180 The principal terpene element within eucalyptus oil is 1, 8-cineole, a cyclic ether and proven enhancer ¹⁸⁸ but its efficacy as an enhancer are mixed. Eucalyptus oil could not enhance the permeation of lipophilic drug like estradiol. Similar results were also reported for the permeation of indomethacin, a lipophilic molecule. The study revealed that oxygen containing terpenes (carvone and 1,8-cineole) were ineffective whereas hydrocarbon terpenes, especially limonene, were effective towards lipophilic drugs. 189 The results of permeation enhancement of ketorolac, a hydrophilic drug also support the above observations towards which oil with oxygen containing terpenes were effective. Eucalyptus oil at 5% produced a flux of 32.47 $\mu g/cm^2/h$, an enhancement of 1.80 times and a T_{lag} of 1.34h. Eucalyptus oil demonstrated maximum permeation rate and shortest T_{lag} (Table 14).

It was therefore appropriate to study the effect of eucalyptus oil concentration on permeation rate of ketorolac. Increasing the concentration of eucalyptus oil to 7.5% and 10% concentration produced a flux of 54.79 and 66.38 μ g/cm²/h, an enhancement of 3.04 and 3.68 times and reduction of T_{lag} to 1.14 h and 1.08h respectively. Further increase in eucalyptus oil concentration was not justified due to skin irritation potential of the oil on prolonged contact with the skin.

Eucalyptus oil at 5%, 7.5%, 10% and 15%w/w concentration produced a flux of 32.47, 54.79, 66.38 and 90.56 μ g/cm²/h, i.e. an enhancement of 1.80, 3.04, 3.68 and 5.02 times and T_{lag} of 1.34, 1.14, 1.08 and 1.11 h respectively. However, the concentration of 15%w/w was not considered due to skin irritation potential. The enhancement in flux of ketorolac across rat skin attained on increasing the concentration of eucalyptus oil is shown in Fig. 28. These essential oils probably modify the solvent nature of the stratum corneum, improving drug partitioning into the tissue. Terpenes are generally good solvents and permeate skin well, 190 with loss of terpenes, from a formulation there could be an alteration of the thermodynamic activity of the permeant. Terpenes may also modify drug diffusivity through the membrane and bring about a reduction of the lag time for permeation, indicating increase in diffusivity of the drug through the membrane following terpene treatment. X-ray diffraction studies have also indicated that d-limonene and 1,8-cineole disrupt stratum corneum bilayer lipids. 191

Table 14 - Permeation data of *in-vitro* studies of hydrogel based transdermal system across rat abdominal skin

PREPARATION ER		FLUX (µg/cm²/h)	Sd	T lag (h)	sd	Q 24 (μg/cm ²)
pH 5.4	pH 5.4 1.00		0.03	7.70	0.47	34.62 (Q 36h)
Ethanol – 10% 3.1:		3.91	0.09	3.26	0.51	80.18
Propanol – 10%	2.85	3.53	0.02	3.68	0.14	71.08
IPA - 10%	3.77	4.67	0.10	3.55	0.28	95.20
Butanol – 10%	2.56	3.18	0.09	3.91	0.10	63.12
Pentanol – 10%	2.18	2.71	0.23	4.28	0.32	52.97
Propylene Glycol 10%	2.59	3.22	0.17	5.68	0.13	57.31
IPA – 15%	6.00	7.44	0.05	3.54	0.34	149.73
IPA – 20%	9.14	11.34	0.06	2.82	0.25	234.26
IPA – 25%	14.55 (1.00)	18.04	0.04	2.71	0.22	377.08
d-limonene – 5%	1.05	18.89	0.62	1.67	0.55	414.89
DMSO – 5%	1.55	27.97	0.99	1.97	0.60	658.43
Transcutol - 5%	1.65	29.72	0.83	2.05	0.10	647.09
Eucalyptus oil – 5%	1.80	32.47	1.63	1.34	0.30	727.52
Eucalyptus oil – 7.5%	3.04	54.79	3.17	1.14	0.34	1242.69
Eucalyptus oil –	3.68	66.38	3.12	1.08	0.32	1509.40
Eucalyptus oil –	5.02	90.56	3.07	1.11	0.40	2055.92
Eucalyptus oil – 10% + pretreatment with abrading gel	5.16	93.10	4.25	1.09	0.32	2115.59

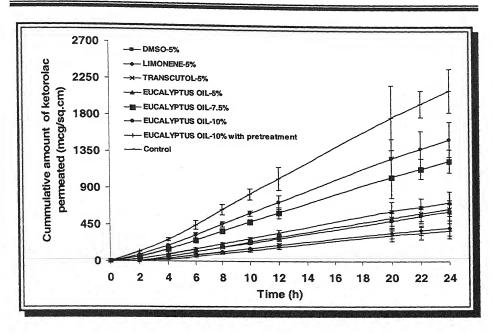


Figure 27 - In-vitro permeation profile of ketorolac through rat abdominal skin from ketorolac transdermal gel system containing ketorolac (2% w/w), HPMC (2% w/w), IPA (25% w/w) and different enhancers formulated using PBS solution of pH 5.4.

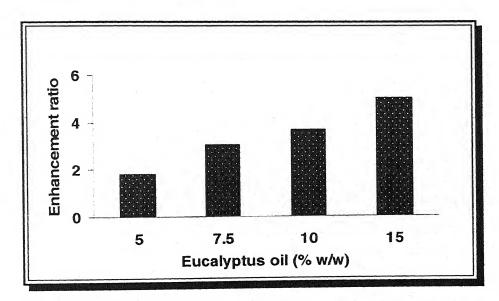


Figure 28 - Enhancement ratio of ketorolac across rat abdominal skin from gel system containing ketorolac (2% w/w), HPMC (2% w/w), IPA (25% w/w) and eucalyptus oil at varying concentrations formulated in PBS solution of pH 5.4.

7.1.4 Effect of abrasion of skin on *in-vitro* skin permeation of ketorolac

Attempts were made to enhance the permeation of ketorolac across skin by abrading the upper skin layer with the help of abrading gel made of crushed apricot seed. There was 5.16 times enhancement in permeation of ketorolac across rat skin from formulation containing ketorolac (2%w/w), HPMC (2%w/w), IPA (25%w/w) and eucalyptus oil (10%w/w) prepared in PBS solution of pH 5.4. The J_{ss} and T_{lag} of 93.10 μ g/cm²/h and 1.09 h were attained respectively after pretreatment.

7.2 HISTOLOGICAL STUDIES

7.3 Effect of formulation on the histology of rat abdominal skin

The control skin (Fig. 29A) showed intact stratum corneum with no swelling of epidermis. No inflammatory cells were seen. The normal skin stratification was intact.

Chemical enhancers dramatically affected the skin. Transdermal formulation containing d-limonene fluidized the inner lipid layers and mild increase in the number of inflammatory cells was observed but the stratum corneum was found to be almost intact (Fig. 28B). Formulation containing DMSO demonstrated fluidization of the inner dermis; the stratum corneum was seen disrupted though inflammatory cells were not observed (Fig. 28C). Transdermal formulation containing eucalyptus oil fluidized the inner dermal

lipids; the skin was found swollen with slight increase in the number of inflammatory cells (Fig. 28D). On treatment of skin with formulation containing transcutol it was observed that only the epidermal portion had swollen with slight fluidization in this part while the basic stratification of the skin was almost maintained (Fig. 28E). The effect of abrading the skin with abrading gel followed by treatment with transdermal formulation containing eucalyptus oil was observed on the skin. Stratum corneum was found disrupted, the skin lipids were vastly fluidized and the skin appeared swollen (Fig. 28F).

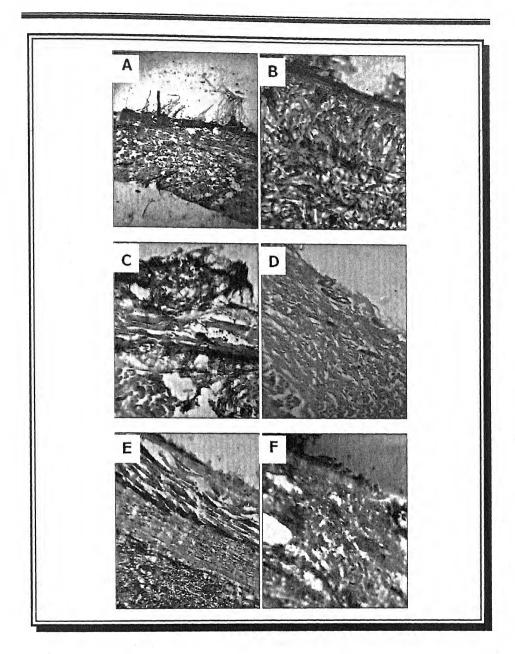


Figure 29 - Histological findings after treatment of rat abdominal skin for 24 h with chemical permeation enhancer gel system observed under microscope after staining with hematoxylin-eosin stain. A - Control, B - d-limonene, C - DMSO, D - Eucalyptus oil, E - Transcutol and F - Application of eucalyptus oil preparation after pretreatment.

7.3 PHARMACOLOGICAL STUDIES

7.3.1 Skin irritation study

The results of the skin irritation studies based on visual observation score suggest that the formulations were safe to be applied on skin. The scores for d-limonene and transcutol were between 0-1; while that for eucalyptus oil and DMSO was between 0-2. (Table 15)

Table 15 - Visual observation score for ketorolac transdermal formulations.

S.No.	Formulation	Visual observation score	± S.D. (n=4)
1	Control	0.25	0.5
2	Limonene-5%	0.25	0.5
3	DMSO-5%	0.5	0.6
4	Transcutol-5%	0	0.0
5	Eucalyptus oil-5%	0.25	0.5
6	Eucalyptus oil-7.5%	0.25	0.5
7	Eucalyptus oil-10%	0.5	0.6
8	Eucalyptus oil-15%	1.5	0.6
9	Eucalyptus oil-10% with pretreatment	0.75	0.5

7.3.2 Acetic acid induced writhing effect (Antinociceptive response)

There was 88.10% inhibition in writhing on oral administration of ketorolac was observed while application of transdermal application without pretreatment and after pretreatment demonstrated 50% and 85.71% inhibition in writhing response (Fig. 30). The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is

thought to be mediated by peritoneal mast cells, ¹⁹² acid sensing ion channels ¹⁹³ and the prostaglandin pathways. ¹⁹⁴ The reservoir type transdermal patch consisting of ketorolac gel thus appears promising in delivering the drug across skin.

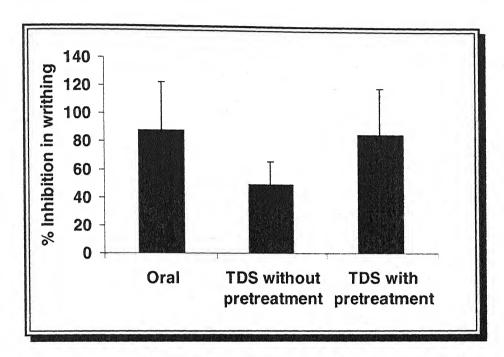


Figure 30 - Antinociceptive response of ketorolac 15 min after induction of writhes with the help of acetic acid (20mg/kg; 2ml/kg) after (i) oral administration of ketorolac and (ii) on application of transdermal patch of ketorolac (TDS) without pretreatment and (iii) after pretreatment with abrasive gel preparation.

7.3.3 Carrageenan induced paw edema

The formulation showed a prominent increase in activity in the carrageenan induced paw inflammation model. Fig. 31 represent the anti-inflammatory activity after oral administration and transdermal application without and after pretreatment with abrading gel.

Ketorolac transdermal patch formulation demonstrated comparable antiinflammatory potential compared to orally administered ketorolac. The antiinflammatory potential was measured in terms of the AUC of graph plotted between difference in paw diameter and time. Compared to the % AUC for untreated paw which was taken as 100%, oral administration showed swelling of 64.04% while transdermal formulation without pretreatment demonstrated 74.16% swelling and after pretreatment swelling appeared to much reduced at 60.67%. The maximum percentage inhibition was observed at 6 h for oral and transdermal formulation without pretreatment and at 4 h after pretreatment. There is clear evidence of enhanced permeation with quicker onset of action for the transdermal formulation applied after pretreatment. This is probably because the primary pathway of transdermally delivered drugs is paracellular, i.e., around the cells, then through the elastin glue. The glue-like compound, elastin is composed of collagen and hyaluronic acid and other lipids, which occupies the interstices between the cells of the top-most layer of the skin (i.e., the epidermis, including - stratum corneum, lucidum, granulosum, spinosus) which must be disrupted in order for the drug, dissolved in a solvent, to be able to transverse through viable skin to the subcutaneous tissues where the cutaneous plexi of the capillary net can be reached. Abrasion of skin by crushed seeds of apricot probably helps in removing if not completely at least partially the upper stratum corneum thus aiding permeation enhancement.

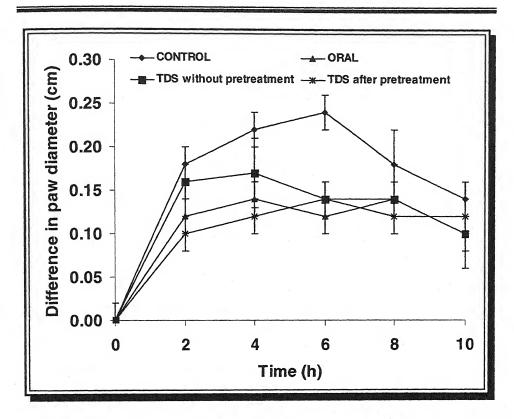


Figure 31 - Anti-inflammatory activity of ketorolac on paw edema induced with carrageenan injection (0.05 ml of 0.5% w/w) in rats (Control) and after oral administration of ketorolac solution (2mg/kg) (Oral) and on application of transdermal patch of ketorolac (TDS) without pretreatment and after pretreatment with abrasive gel preparation.

RESULTS AND DISCUSSION

MICROEMULSION GEL BASED
TRANSDERMAL DELIVERY SYSTEM
OF DEXAMETHASONE

8. RESULTS AND DISCUSSION – MICROEMULSION GEL BASED TRANSDERMAL DELIVERY SYSTEM OF DEXAMETHASONE

8.1 PRE-FORMULATION STUDIES FOR PREPARATION OF DEXAMETHASONE FORMULATION

8.1.1 Pseudoternary phase diagram study

The aim of construction of pseudo-ternary phase diagrams was to find out the existence range of microemulsions. The phase diagrams of the four selected systems are presented in Fig. 32. It was observed that oil/water (o/w) microemulsion region was slightly larger for olive oil and nutmeg oil, while linseed oil and almond oil had comparatively smaller areas. However all ternary phase diagrams revealed that at least 10% w/w oil phase could be incorporated using egg lecithin and IPA as surfactant and cosurfactant respectively. The dark shaded region in the phase diagram marks the clear transparent microemulsion phase.

There was occurrence of distinct phases, the immiscible phase, the translucent phase and the clear transparent phase. The conversion from turbid phase to oil-in-water (o/w) microemulsions phase was observed. The major clear

region on the phase diagram represents the non-existent or turbid and conventional emulsion. The microemulsion region changed slightly in size with the increasing ratio of surfactant to cosurfactant. It was observed that the amount of oil that could be incorporated varied with the type of oil and also on the ratio of S:CoS. The S:CoS ratio yielding largest areas was selected for each oil.

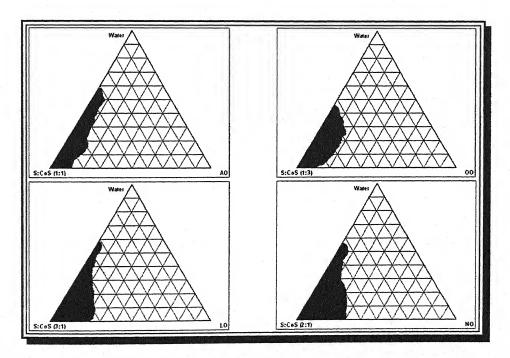


Figure 32 - Pseudo-ternary phase diagrams of the oil, surfactant-water system at the 1:7:2 weight ratios of oil, egg lecithin-IPA mixture and distil water at 25°C (Shaded portion represents microemulsion region).

Since the order of the addition of the ingredients has significant influence on the preparation of microemulsions, ^{195,196} therefore dexamethasone was first dissolved/dispersed in the oil phase and to it was added S:CoS mixture of

selected ratio at defined concentrations and then slowly titrated with water.

The concentrations of the components are represented in Table 6.

8.1.2 Screening of oils for microemulsions

In order to develop microemulsion formulations for dermal delivery of poorly water-soluble dexamethasone, the optimum oil was selected by determining the concentration of dexamethasone that would dissolve. Four different microemulsions were prepared with oil, S:CoS (egg lecithin-IPA) and distilled water in 1:7:2 ratio. The solubility of dexamethasone in microemulsions of various oils is reported in Table 16.

Table 16 - Solubility of dexamethasone in microemulsions of various oils at 25 $^{\circ}$ C (mean \pm S.D., n=4).

Microemulsion oil (S:CoS)	Solubility (mg/ml)
Almond oil (1:1)	10.63 ± 0.11
Olive oil (1:3)	14.27 ± 0.36
Linseed oil (3:1)	9.49 ± 0.22
Nutmeg oil (2:1)	11.89 ± 0.47

The solubility of dexamethasone was highest in microemulsions of olive oil followed by nutmeg oil, almond oil and linseed oil. The solubility of dexamethasone in microemulsions of various oils decreased slightly when compared to that in olive oil. The addition of egg lecithin and IPA to oil probably would positively influence drug solubility. It was therefore inappropriate to study the solubility of dexamethasone in oil phase.

Microemulsions were therefore chosen for assessing the solubility. Dexamethasone demonstrated substantial solubility in microemulsions of various oils. As per the observed solubility olive oil and nutmeg oil would be the most appropriate oils for the development of microemulsion. In order to verify the selection of oil the *in-vitro* skin permeation rate of dexamethasone from the microemulsions containing dexamethasone, oil, egg lecithin-IPA mixture and water was also determined.

8.2 CHARACTERIZATION OF DEXAMETHASONE LOADED MICROEMULSION PREPARATION

The results of the characterization studies are presented in Table 17.

8.2.1 Droplet size determination of microemulsion

The smallest droplet size of all microemulsions ranged from 4 - 13 nm. The droplet size of microemulsion was found to vary with the oil. Olive oil and nutmeg oil produced microemulsions of 10 - 13 nm diameter while almond oil and linseed oil microemulsions had droplet size of 4 - 6 nm. The small droplet sizes are very much a prerequisite for drug delivery as the oil droplets tend to fuse with the skin thus providing a channel for drug delivery.

8.2.2 Viscosity measurements of microemulsion based hydrogel

The viscosities of the microemulsion based carbopol gels ranged from 160 to 200 Poise for microemulsion gel.

8.2.3 Conductivity measurements of microemulsion

The conductivities were in the range of 8 X 10⁻⁶ to 10 X 10⁻⁶ S/cm. The conductivities of the microemulsion revealed that water was the external phase and the microemulsion was o/w type.

8.2.4 Stability studies

On subjecting the microemulsions to centrifugation for 15 min at 3000 rpm the formulations did not show any phase separations.

On subjecting to thermal cycling between 4°C and 45°C with storage at each temperature for at least 48 h, the formulations were found to be stable. The clarity of the formulations was not compromised on returning to room temperature.

The microemulsion was gelled by addition of carbomer 934 followed by addition of triethanolamine it was observed that the microemulsion structure was disturbed. However, a clear gel could be obtained by omitting triethanolamine. The microemulsion-based hydrogel with 1.0% w/w carbopol were stable at 40°C. No phase separation and degradation was observed during three months. The microemulsion based hydrogel with 1.0% w/w carbopol formulated as reservoir type transdermal device could be applied to the skin as such or after treating the area of the skin with abrading gel.

8.2.5 pH measurements

The microemulsion formulations had pH values varying from 4.18 to 4.73. Incorporation of different oils did not much affect the pH of the microemulsions. The centrifuge test showed that the microemulsion had good physical stability.

Table 17 - Physicochemical parameters of tested dexamethasone loaded microemulsions.

Microemulsion oil	Droplet size (nm)	Viscosity (Poise)	Conductivity (S/cm, 10 ⁻⁶)	рН
Almond oil (AO)	4.27	172.5	9.1	4.52
Olive oil (OO)	10.8	162.9	8.9	4.18
Linseed oil (LO)	5.02	193.8	8.2	4.46
Nutmeg oil (NO)	12.7	166.3	9.6	4.73

8.3 IN-VITRO SKIN PERMEATION STUDIES OF MICROEMULSION BASED DEXAMETHASONE GEL PREPARATIONS

8.3.1 *In-vitro* skin permeation studies of dexamethasone across rat skin

The *in-vitro* permeation profiles of microemulsion based dexamethasone formulations applied without pretreatment with abrading gel and after treating are presented in Fig. 33 and Fig. 34 respectively. Flux observed for formulation OO and NO formulations was $40.76 \, \mu g/cm^2/h$ and $36.02 \, \mu g/cm^2/h$ respectively. The high permeation rate of microemulsions might be

attributed to several factors. Microemulsions could act as drug reservoirs where drug is released from the inner phase to the outer phase and then further onto the skin. Secondly, due to the small droplet size, droplets settled down to close contact with the skin and a large amount of oil phase in microemulsions might penetrate into skins. 197 In addition, due to the small droplet diameters of microemulsions, the likely mechanism may also be the permeation of dexamethasone directly from the droplets into the stratum corneum without microemulsion fusion to the stratum corneum and subsequent permeation enhancement. The concentration of the mixtures of egg lecithin and IPA was 70% w/w in all preparations but their ratio probably influenced the permeation rates. These microemulsion formulation OO and NO had S:CoS ratio of 1:3 and 2:1 respectively. Despite the fact that formulation NO had larger droplet size its permeation rate was not much affected, a phenomenon which needs to be analyzed. Microemulsion gel based system of almond oil and linseed oil had lower flux i.e. 28.91 and 21.80 µg/cm²/h respectively. Isopropyl alcohol acts as permeation enhancers and has strong permeation enhancing effect. It could enhance the solubility of dexamethasone in the skin by disrupting the lipid bi-layer of the skin.

The lecithin and IPA present act not only as surfactant and stabilize the microemulsion but they also act as permeation enhancers. They probably interact with the intercellular lipids in the stratum corneum. IPA tends to fluidize the lipids thus increasing the permeation of dexamethasone.

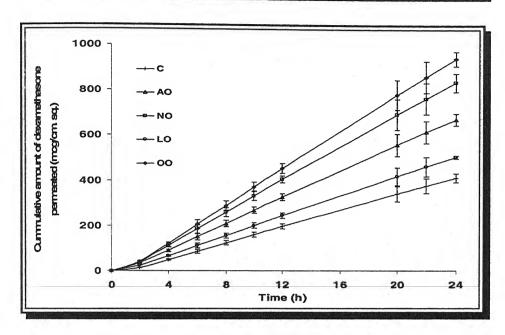


Figure 33 - Permeation profiles of dexamethasone through excised rat skins from microemulsions based gel formulations of different oils without pretreatment.

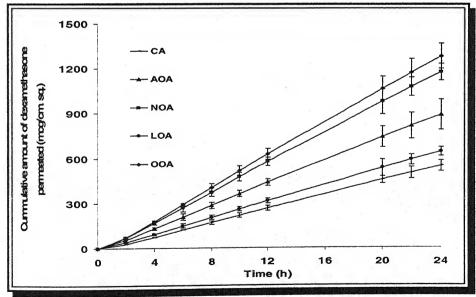


Figure 34 - Permeation profiles of dexamethasone through excised rat skins from microemulsions based gel formulations of different oils after pretreatment.

It was observed that use of abrading gel significantly increased the permeation rate of dexamethasone across rat skin (p<0.05). The use of abrading hydrogel of crushed apricot seed aids in enhancing the drug permeation by removing the upper dead layer of skin i.e. stratum corneum. There was 1.31 to 1.39 times increase in the permeation of dexamethasone across rat skin after abrading the skin compared to the same formulation when applied without pretreatment. The flux of dexamethasone formulation NOA increased from 36.02 to $50.24 \,\mu g/cm^2/h$, while that of formulation OOA increased from 40.76 to $54.98 \,\mu g/cm^2/h$. Formulation OO showed an enhancement ratio of 2.25 without pretreatment as compared to 1.20, 1.60 and 1.99 for LO, AO and NO. The enhancement ratios and permeability coefficient values are reported in the Table 18.

Table 18 - Permeation parameters of the dexamethasone loaded microemulsions based gel transdermal system.

Microemulsion formulation code	Enhancement ratio (ER)	Flux (J _{ss}) µg/cm²/h	S.D. (n=4)	Lag time (T _{lag}) h	S.D. (n=4)
С	1.00	18.10	0.68	1.17	0.31
CA	1.31	23.70	0.40	0.79	0.36
AO	1.60	28.91	1.06	0.72	0.29
AOA	2.09	37.91	0.84	0.43	0.31
00	2.25	40.76	1.64	0.89	0.32
OOA	3.04	54.98	1.08	0.62	0.35
LO	1.20	21.80	1.38	0.80	0.48
LOA	1.52	27.49	0.71	0.51	0.33
NO	1.99	36.02	1.24	0.79	0.34
NOA	2.78	50.24	1.04	0.53	0.33

A comparison of the enhancement ratios of formulations applied without pretreatment and after pretreatment is shown in Table 19. Abrasion of skin demonstrated an enhancement ranging from 1.26 to 1.39 as seen in diagonal relationship between unabraded and abraded enhancement ratios.

Table 19 - Enhancement ratios of various transdermal formulations of dexamethasone with respect to each other.

Formulation	C	AO	00	LO	NO
CA	1.31	0.82	0.58	1.09	0.66
AOA	2.09	1.31	0.93	1.74	1.05
OOA	3.04	1.90	1.35	2.52	1.53
LOA	1.52	0.95	0.67	1.26	0.76
NOA	2.77	1.74	1.23	2.30	1.39

Maximum flux of $54.98~\mu\text{g/cm}^2\text{/h}$ was achieved for formulation OOA after pretreatment with abrading gel and the total amount of drug delivered across

rat skin was 1283.54 μ g/cm²/h . The observed lag time was reduced from 0.89 to 0.62 h after pretreatment of skin. There was significant (p<0.05) reduction of lag time on pretreatment of skin with abrading hydrogel.

This may be probably because the primary pathway of transdermally delivered drugs is paracellular, i.e., around the cells, then through the elastin glue. The glue-like compound, elastin, composed of collagen and hyaluronic acid and other lipids, which occupies the interstices between the cells of the top-most layer of the skin (i.e., the epidermis, including - stratum corneum, lucidum, granulosum, spinosus) must be dissolved and/or disrupted in order for the drug, to be able to transverse through viable skin to the subcutaneous tissues where the cutaneous plexi of the capillary net can be reached. Permeation of oil into the lipid layers of the skin may lead the change in the lipid barrier properties. Further disruption of stratum corneum barrier by the abrading agent might enhance the permeation by removing the upper dead stratum corneum.

In case of human subjects it is desirable that the formulation delivers 0.5 to 9 mg of dexamethasone in 24 h. Therefore employing a patch of size 6.8 cm², the desired plasma levels can be achieved.

The main factors determining the transdermal permeation of drugs are the mobility of drug in the vehicle, release of drug from the vehicle, and permeation of drug into the skin. These factors affect either the thermodynamic activity that drives the drug into the skin or the permeability

of drug in the skin, particularly stratum corneum. Microemulsions improve the transdermal delivery of several drugs over the conventional topical preparations such as emulsions^{198,199} and gels.^{200,201} Mobility of drugs in microemulsions is more facile, as compared to gels which increase the viscosity and further decrease the permeation in the skin.²⁰² The superior transdermal flux from microemulsions has been shown to be mainly due to their high solubilization potential for lipophilic and hydrophilic drugs. This generates an increased thermodynamic activity towards the skin.

Microemulsions may affect the permeability of drug in the skin. In this case, the components of microemulsions serve as permeation enhancers. Several compounds used in microemulsions have been reported to improve the transdermal permeation by altering the structure of the stratum corneum. For alkanols widely used as permeation example. short chain are enhancers. 203,204,205 It is known that oleic acid, a fatty acid with one double bond in the chain structure, perturbs the lipid barrier in the stratum corneum by forming separate domains which interfere with the continuity of the multilamellar stratum corneum and may induce highly permeable pathways in the stratum corneum. 206,207,208 Apart from chemical enhancers, isopropyl myristate (IPM) is also used as a permeation enhancer in transdermal formulations, but the mechanism of its action is poorly understood.²⁰⁹ Nonionic surfactants are widely used in topical formulations as solubilizing agents but some recent results indicate that they may also affect the skin barrier function.²¹⁰ It is of interest to explore the effects of these components in the organized microemulsion structures. A unique attempt was made to emulsify coconut oil with the help of polyoxyethylene 2-cetyl ether (Brij 52) and isopropanol or ethanol, forming stable isotropic dispersion thus paving way for use of vegetable oil to be used as oil phase in microemulsion.²¹¹

8.4 PHARMACOLOGICAL STUDIES

8.4.1 Skin irritation study

The skin irritation studies revealed that all formulations were non-sensitizing and safe for use. The visual observation scores are presented in Table 20.

Table 20 - Visual observation score for microemulsion gel based transdermal dexamethasone formulation.

S.No.	Formulation	Visual observation score	± S.D. (n=4)
1	С	0.00	0.00
2	AO	0.00	0.00
3	00	0.00	0.00
4	LO	0.25	0.50
5	NO	0.00	0.00
6	CA	0.00	0.00
7	AOA	0.00	0.00
8	OOA	0.25	0.50
9	LOA	0.50	0.58
10	NOA	0.00	0.00

8.4.2 In vivo anti-inflammatory studies

The microemulsion based transdermal system showed a significant response in the carrageenan inflammation model. The anti-inflammatory activity of the microemulsion based transdermal system in carrageenan induced paw edema model. The formulation demonstrated significantly improved activity compared to that of the control (p<0.05), improvement was measured in terms of paw edema volume 4 h after carrageenan injection.

The anti-inflammatory potential revealed that that there was a significant reduction of paw swelling when compared to the results from the non abraded skin. There was 62.22 and 73.65% reduction of paw volume for microemulsion gel based transdermal device for OOA and NOA respectively when applied after pretreatment of the site of application with abrading gel. NOA demonstrated a significant reduction (p<0.05) in paw edema compared to OOA.

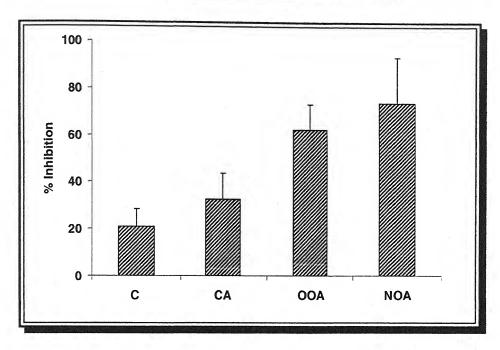


Figure 35 - Anti-inflammatory activity of microemulsion based gel of dexamethasone.

Comparative reductions in edema volume with respect to control are shown in Fig. 35. A 32.73% decrease in paw inflammation was recorded when the control formulation was applied dermally after abrading the skin with abrading gel, thus clearly indicating the role of abrading gel in enhancing permeation. The effect of nutmeg oil over that of olive oil was more pronounced. NOA based microemulsion formulation demonstrated 11.73% higher activity as compared to olive oil based microemulsion formulation. The transdermal microemulsion gel based system thus has due potential to deliver dexamethasone at therapeutically effective concentrations.

RESULTS AND DISCUSSION

PRONIOSOME BASED TRANSDERMAL SYSTEM OF PIROXICAM

9. RESULTS AND DISCUSSION - PRONIOSOME BASED TRANSDERMAL SYSTEM OF PIROXICAM

9.1 EVALUATION

Among the niosomes prepared with spans, S4 and S6 showed maximum percentage entrapment i.e. 90.4 and 94.8% respectively. The particle size analysis revealed that niosomes from S4 were larger as compared to those of S6 (Fig. 36). Vesicles with smaller diameter are believed to better permeate through the skin as smaller vesicles tend to fuse readily. Niosomes of S6 were smaller in size, demonstrated higher entrapment efficiency and higher surface area as compared to that of S4. Niosomes of S2 demonstrated low entrapment efficiency and was not taken up for further study. It was observed that niosomes of Span 40 produced niosomes of larger size but in case of piroxicam vesicles from Span 60 had higher entrapment efficiency and were therefore selected. This probably could be credited to its high transition temperature and low permeability. Useful correlation between vesicle size and permeation have been reported by Gabrijelcic et. al., 1994²¹² and Esposito et. al., 1998.213 for multi lamellar vesicles of hydrophilic drugs formed by reverse-phase evaporation. It was observed by them that size and permeability were inversely related. It was observed by Du Plessis, et. al., 1994²¹⁴ that an average vesicle size of 300 nm was suitable for percutaneous drug delivery. Verma, et. al., 2003²¹⁵ evaluated the effect of size on dermal delivery of carboxyfluorescein and reported maximum delivery for small sized particle of 120 nm and least for particles of size 810 nm. The results of *in-vitro* studies were confirmed by confocal scanning laser microscopy. The permeation enhancement decreased as the particle size increased. It is widely reported in literature that larger vesicles may not permeate into the deeper layers of the skin and stay on or into the SC thus further strengthening the barrier properties of the skin. They may form a layer on the skin and reduce permeation.

Attempt was also made to incorporate lecithin to influence entrapment efficiency. Researchers have however attempted to formulate piroxicam into self-emulsifying lipospheres using homolipid of *Capra hircus*. Lecithin also possesses antioxidative potential that depends directly on the content of phospholipids. Egg lecithin with higher phospholipid content exerts better antioxidative properties. Furthermore, due to its specific physico-chemical properties, amphiphility in particular, the phospholipid the largest portion of the lecithin lipids, forms enclosed membrane systems in the presence of water.²¹⁷

Egg lecithin was found to enhance drug entrapment to 96.1%. Incorporation of lecithin is also justified as it acts as permeation enhancers.

In the present study attempt was also made to formulate niosomes from proniosomes formed on maltodextrin and sorbitol. Preparing proniosomes on maltodextrin and sorbitol carriers provided a base for the surfactant solution a slight increase in entrapment (Fig. 37) was observed i.e. 97.2 and 98.6% respectively. It was observed that preparing proniosomes on dry powder base was easier, provided the powder is not over wetted during the process. Preparing proniosomes on maltodextrin was comparatively easy as compared to sorbitol but it was necessary that the solution be incorporated in very small amounts and complete drying be ensured before further additions are made.

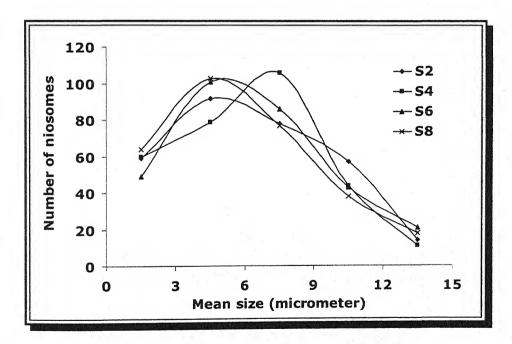


Figure 36 - Mean size distribution of piroxicam niosomes prepared from S2, S4, S6 and S8.

Maltodextrin is a polysaccharide; it has minimal solubility in organic solvents. It is widely used for micro encapsulation. Thus, it is possible to coat maltodextrin particles by simply adding surfactant in organic solvent to dry maltodextrin and evaporating the solvent. The maltodextrin particle morphology is preserved (Fig. 38C), circular maltodextrin particles can be used for a significant gain in surface area. The higher surface area results in a thinner surfactant coating, which makes the re-hydration process more efficient. The use of maltodextrin as the carrier in the proniosome preparation permitted flexibility in the ratio of surfactant and other components which can be incorporated.

Coating sorbitol results in a solid cake like mass (Fig. 38E). It was necessary that the sorbitol bed be completely dry before further additions are made and making proniosomes with a reduced amount of sorbitol was not only tedious but lead to niosomes with larger vesicle size. Niosomes formed from conventional proniosomes, maltodextrin based and sorbitol based proniosomes are shown in (Figure 38B, 38D and 38F) respectively.

Proliposomes containing varying amount of nicotine, a model drug, were prepared using sorbitol and lecithin by Chung, 1999.²²⁰ and the release pattern of nicotine was apparently similar to that of the Exodus[®] patch, a commercially available transdermal nicotine formulation.

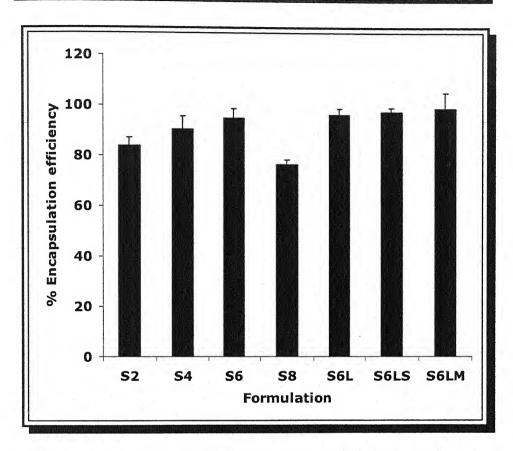


Figure 37 - Encapsulation efficiency of various piroxicam niosomes (%EE±S.D.).

Monomers of amphiphiles organize into bilayers to form lipid vesicles. Such bilayers are unstable or leaky and hence cholesterol is a common ingredient included to stabilize them. Cholesterol stabilizes bilayers, prevents leakage, and retards permeation of solutes enclosed in the aqueous core of these vesicles. However other lipids like polyglyceryl-3-di-isostearate and polysorbate-80 have also been attempted.²²¹

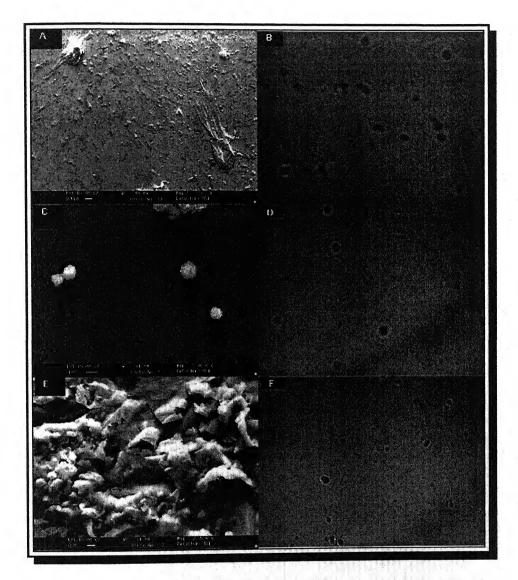


Figure 38 - SEM images of proniosomes and polarized microscopic view of niosomes (40x): conventional (A & B), maltodextrin based (C & D), sorbitol based (E & F).

9.2 IN-VITRO SKIN PERMEATION STUDIES OF PIROXICAM GEL PREPARATIONS

9.2.1 In-vitro permeation studies of piroxicam across rat skin

The amount of drug permeated across rat abdominal skin was determined for piroxicam carbopol gel and niosomes formulated using span 60 and lecithin (S6L) and on maltodextrin (S6LM) and sorbitol as base (S6LS) is shown in Fig. 39. The flux attained at the end of 24 h was 4.82, 35.61, 20.14 and 19.05 ug/cm²/h respectively and their enhancement ratios were 1.00, 7.39, 4.18 and 3.95 respectively. The incorporation of maltodextrin and sorbitol retarded drug release. Researchers in South Korea formulated proliposomes containing varying amount of nicotine, using sorbitol and lecithin. Microscopic observation revealed that this preparation is converted to liposomes almost completely within minutes following contact with water.²²¹ These potential lipid vesicles have been postulated as an effective means of drug delivery across skin^{222,223} it is therefore not surprising that many drugs have been successfully formulated into lipid vesicles as frusemide, 224 hydrocortisone and dexamethasone, 225 melatonin, 226 estradiol 227 sodium ascorbyl phosphate, 228 adriamycin.²³⁰ diclofenac,²³¹ levonorgestrel, 232, ethinvlestradiol²²⁹ zidovudine, 233 hepatitis B surface antigen, 234 propranolol hydrochloride, 155,235 nimesulide, 236 ketoprofen 237 and cyclosporin A. 238

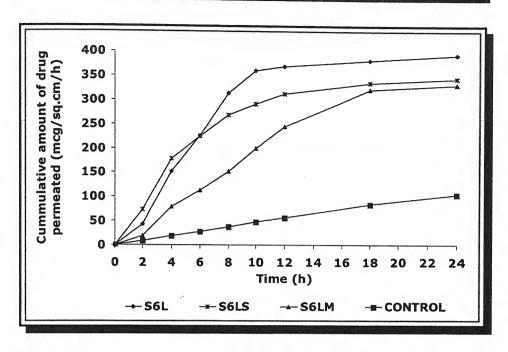


Figure 39 - Cumulative amount of drug permeated through rat abdominal skin from different piroxicam noisomal gel.

9.3 PHARMACOLOGICAL STUDIES

9.3.1 Skin irritation study

The results of the skin irritation studies based on visual observation score suggest that the formulations were safe to be applied on skin. (Table 21)

Table 21 - Visual observation score for piroxicam transdermal formulations.

S.No.	Formulation	Visual observation score	\pm S.D. (n=4)
1	S6L	0.25	0.5
2	S6LM	0.25	0.5
3	S6LS	0.5	0.6
4	Control gel	0	0.0

9.3.2 Carrageenan induced paw edema

The results of the anti-inflammatory studies revealed that span 60 based lecithin vesicles (S6L) showed maximum inhibition in paw swelling (Fig. 40). Percent inhibition was found to be 75.69%, 40.58%, 67.59% and 16.60% for S6L, S6LS, S6LM and control gel respectively. It is probable that there is enhanced drug delivery from lipid vesicles. The short fall seen with maltodextrin and sorbitol based formulations account for the slow release observed in in-vitro studies.

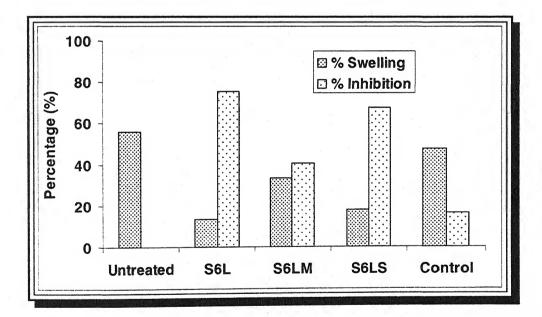


Figure 40 - Anti-inflammatory studies of proniosomal gel based piroxicam transdermal system



10. COMPARATIVE EVALUATION

10.1.1 Permeation barriers

Formulations containing different drugs viz. ketorolac, dexamethasone and piroxicam were subjected to *in-vitro* permeation studies across shed snake skin and human cadaver skin. The shed snake skin (*Naja Naja Khaotia*) was treated with phosphate buffer saline pH 7.4 overnight while the adhering fat was carefully removed off the human cadaver skin before mounting on the vertical diffusion cell. The flux and lag time attained are reported in Table 22. The flux attained across rat abdominal skin, human cadaver skin and shed snake skin for optimized hydrogel based transdermal system of ketorolac was found to be 66.38, 74.89 and 52.13 µg/cm²/h. T_{lag} was found to be 1.07, 1.46 and 1.20 h and the *in-vitro* permeation profile are shown in Fig. 41, Fig. 42 and Fig. 43 respectively.

The flux attained across rat abdominal skin, human cadaver skin and shed snake skin for optimized microemulsion based transdermal system of dexamethasone was found to be 54.98, 42.65 and 44.96 μ g/cm²/h. T_{lag} was found to be 0.61, 1.41 and 0.85 h.

The flux attained across rat abdominal skin, human cadaver skin and shed snake skin for optimized proniosome based transdermal system of piroxicam was found to be 38.34, 32.90 and 23.40 μ g/cm²/h. T_{lag} was found to be 0.24, 0.38 and 0.31 h.

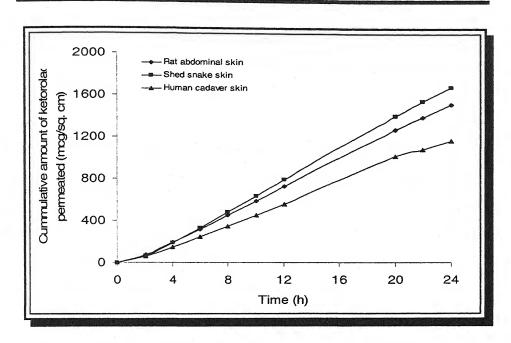


Figure 41 - *In-vitro* permeation profile of hydrogel based transdermal system of ketorolac across different permeation barriers

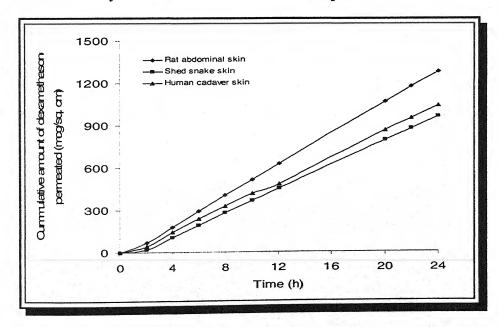


Figure 42 - *In-vitro* permeation profile of microemulsion gel based transdermal system of dexamethasone across different permeation barriers

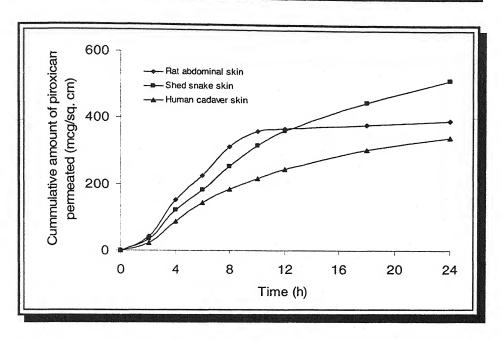


Figure 43 - *In-vitro* permeation profile of proniosomal gel based transdermal system of piroxicam across different permeation barriers

Shed snake skin was employed as permeation barrier as the total lipid content in shed snake skin is similar to that of human stratum corneum, ^{239,240} the skin bears the similar structure in that it contains multi-lamellar lipid sheets that exist in intermediate mesolayers between cornified cells. ²⁴¹ The snake skin seems quiet a suitable model membrane for *in-vitro* drug permeation studies. The *in-vitro* permeation of different formulations was studied across shed skin of snake Cobra (*Naja Naja Khaotia*) as model permeation barrier. ²⁴² Shed snake skin is easily available in large quantity as snakes periodically shed their skin as part of the natural process. The suitability of shed snake skin as

barrier requires to be established so that it may be employed in permeation studies. However human cadaver skin is the best suited permeation barrier as the formulation is intended for human application. But the difficulty of procuring the skin is a major drawback. Further the skin should be used immediately or requires being stored in deep freezers. The shed snake skin is free of such limitations. A single snake shed skin can provide multiple samples, be stored at room temperature and can be easily transported. The application of snake shed skin as barrier is further postulated as the lipid content of both snake shed skin and human cadaver skin are similar. It has been proposed that it is the lipid content that plays a vital role as permeation barrier rather than the lipid composition.²⁴³ However a look at the lipid components shows that ceramides constitute the major lipids of human cadaver skin while shed snake skin largely contains phospholipids. 242, 243 A number of hydrophilic and lipophilic drugs have been evaluated thus ascertaining the suitability of shed snake skin as permeation barrier,244 though others have also postulated against it.²⁴⁵

Table 22 - Comparison of *in-vitro* permeation parameters for different permeation barriers

	Permeation parameters						
Permeation barrier	Drug employed - Ketorolac						
	Flux	Q ₂₄	Tlag	K _p x 10 ⁻³	D	Px10 ⁻³	
Rat abdominal skin	66.38	1509.40	1.07	35.88	36.94	14.96	
Human cadaver skin	74.89	1162.24	1.46	40.48	37.81	19.48	
Shed snake skin	52.13	1674.24	1.20	28.18	48.57	10.85	
Permeation	Drug employed - Dexamethasone						
barrier	Flux	Q ₂₄	T_{lag}	Kp x 10 ⁻²	D	Px10 ⁻²	
Rat abdominal skin	54.98	1283.54	0.61	14.86	64.80	3.53	
Human cadaver skin	42.65	1039.67	1.41	11.53	39.15	5.36	
Shed snake skin	44.96	961.65	0.85	12.15	68.57	3.31	
Permeation	Drug employed - Piroxicam						
barrier	Flux	Q ₁₂	T_{lag}	Kp x 10 ⁻³	D	Px10 ⁻³	
Rat abdominal skin	35.61	359.89	1.09	17.65	36.26	7.50	
Human cadaver skin	27.93	317.35	1.23	15.10	44.88	6.12	
Shed snake skin	19.95	218.59	1.16	10.78	50.24	4.01	

The order probably is observed on account of the thickness of the chief rate limiting barrier i.e. stratum corneum. The reported thickness of rat SC is 15.4 while that of human SC is $18.2 \, \mu m$. 176

The probable reason for the slow flux across shed snake skin is due to either difference in lipid composition or lack of hair follicles (Fig. 44 and 45) and water content in shed snake skin. The human skin comprises of lipids 20%, a proteins 50%, β proteins 20% and non fibrous proteins 10% while shed snake skin comprises of cholesterol 27%, cholesteryl 10%, fatty acids 9%, cholesteryl sulphate 1.9%, ceramide esters 3.8% and sterol diesters 0.9%. More over the shed snake skin does not support transfollicular pathway for drug permeation. The unsuitability of shed snake skin has also been put forth by Rigg and Barry, 1990.²⁴⁵ Our results also support the view that the shed snake skin's use as a permeation barrier is not justified. An almost linear cure is in terms of flux achieved. The shed snake showed the least flux in all cases followed by human cadaver skin and the rat abdominal skin. No such trend was observed in lag time. Reduced T_{lag} was observed for dexamethasone, a lipophilic drug towards human cadaver skin while piroxicam showed enhanced lag time with human cadaver skin.

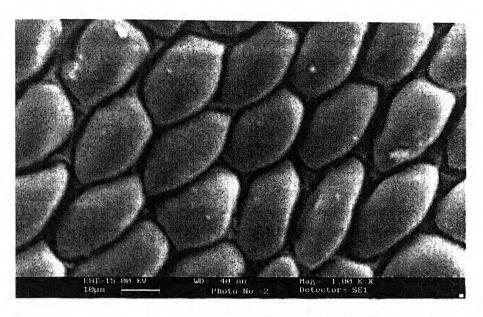


Figure 44 - SEM view of dorsal outer side of shed snake skin

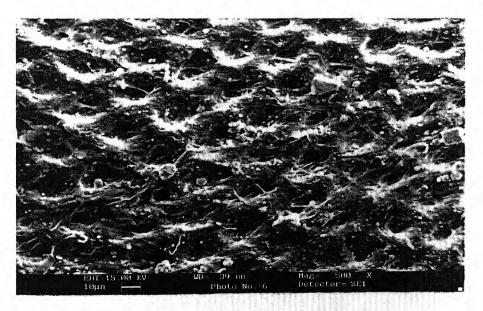


Figure 45 - SEM view of dorsal inner side of shed snake skin

10.1.2 Drug stability studies

Stability studies were carried out according to ICH guidelines²⁴⁶ to establish the structural integrity of the reservoir formulation. The studies revealed no significant changes in the physical appearance of the reservoir formulations.

The degradation constant of transdermal formulations of ketorolac, dexamethasone and piroxicam were found to be 2.3×10^{-4} days, 2.07×10^{-5} days and 1.84×10^{-5} days respectively (Fig. 46).

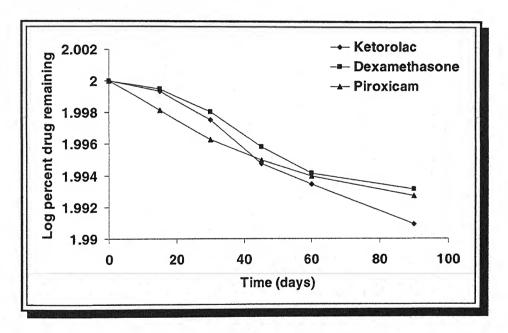


Figure 46 - Log percent drug remaining against time plot for transdermal formulations

Since there was no significant change in drug content (less than 5%), a tentative shelf life of 24 months may be assigned to the formulations.

10.1.3 Model fitting

In-vitro permeation data were fitted to different equations and kinetic models to explain the release kinetics of various transdermal formulations.

Table 23 - Coefficient of correlation (r)

Drug	Rat abdominal skin		Shed snake skin		Human cadaver skin	
Release	Zero order	First order	Zero order	First order	Zero order	First order
Ketorolac	0.9977	0.9931	0.9958	0.9896	0.9969	0.9936
Dexamethasone	0.9990	0.9820	0.9953	0.9842	0.9983	0.9870
Piroxicam	0.9683	0.9668	0.9635	0.9627	0.9646	0.9628

Table 24 - Coefficient of determination (r²)

Drug	Rat abdominal skin		Shed snake skin		Human cadaver skin	
Release	Zero order	First order	Zero order	First order	Zero order	First order
Ketorolac	0.9954	0.9862	0.9916	0.9793	0.9938	0.9872
Dexamethasone	0.9980	0.9643	0.9906	0.9686	0.9966	0.9742
Piroxicam	0.9375	0.9346	0.9283	0.9268	0.9304	0.9270

The best fit with highest coefficient of correlation (r) (Table 23) and coefficient of determination (r²) (Table 24) were computed. Zero order release was observed in all cases.



11. SUMMARY AND CONCLUSION

HYDROGEL BASED TRANSDERMAL DRUG DELIVERY SYSTEM OF KETOROLAC

The objective of the present study was to formulate hydrogel based formulation and to design a reservoir type transdermal patch for delivery of ketorolac a potent analgesic agent and assess its feasibility for transdermal application. The low permeability of skin is the rate-limiting step for delivery of most of the drugs.

Studies were carried out to investigate the effect of ionization, solvents and enhancers on the permeation of ketorolac from hydrogels through rat abdominal skin. The reservoir type transdermal patch was fabricated and the core was filled with gel system of a non ionic polymer HPMC.

The formulations were subjected to *in-vitro* permeation studies, skin irritation studies and stability studies. An increase in the permeation of unionized fraction of ketorolac was observed as pH decreased. Effect of various alcohols on permeation rate revealed IPA as most promising. An increase in the concentration of isopropyl alcohol led to enhanced permeation of ketorolac with highest flux being attained at 25% w/w concentration.

Various permeation enhancers viz. dimethyl sulphoxide, d-limonene, eucalyptus oil and transcutol (diethylene glycol monoethyl ether) were incorporated into the gel system. Permeation enhancement of ketorolac with

different enhancers followed the order eucalyptus oil> transcutol> DMSO> d-limonene.

Eucalyptus oil showed maximum flux of $66.38~\mu g/cm^2/h$. Further enhancement of flux was achieved by using a well known but not yet employed abrasion technique. Abrasion was effected by rubbing an abrasion gel comprising of apricot crushed seeds in HPMC hydrogel base. From the results, eucalyptus oil/IPA/phosphate buffer saline pH 5.4 solvent systems can be considered effective in augmenting skin permeation, with potential applications in transdermal delivery of the drug. There was 5.16 times enhancement in flux at $93.10~\mu g/cm^2/h$ was attained.

Dermal administration of ketorolac gel to rats showed that the formulation was capable of delivering the drug across skin in vivo at an enhanced rate. A reservoir type transdermal patch for delivery of ketorolac thus appears to be feasible of delivering ketorolac across skin.

MICROEMULSION BASED DRUG DELIVERY SYSTEM OF DEXAMETHASONE

The study was carried out to formulate microemulsion based hydrogel formulation for transdermal delivery of dexamethasone. Oils of natural origin and potential beneficial/therapeutic value viz. almond oil, olive oil, linseed oil and nutmeg oil were screened as the oil phase. Microemulsions based system was chosen due to its good solubilizing capacity and skin permeation capabilities. The pseudoternary phase diagrams for microemulsion regions

were constructed using various oils, egg lecithin as the surfactant, isopropyl alcohol as the cosurfactant and distilled water as aqueous phases. Microemulsion gel formulations were prepared and filled into reservoir type transdermal system. The ability of various microemulsion formulations to deliver dexamethasone through rat skin was evaluated in-vitro using Keshary Chien diffusion cells. In order to enhance permeation the skin was treated with an abrading gel (crushed apricot seed in hydrogel base). The in-vitro permeation data showed that microemulsions increased the permeation rate of dexamethasone compared to control. The optimum formulation consisting of 0.1% dexamethasone, 10% olive oil, 70% egg lecithin: IPA (2:1) and water showed a permeation rate of 54.98 µg/cm²/h. The studied microemulsionbased hydrogel were stable and non-irritating to skin. The pharmacodynamic studies indicated that microemulsion based on nutmeg oil demonstrated significantly (p<0.05) higher anti-inflammatory potential. Nutmeg oil based transdermal microemulsion gel system demonstrated 73.65% inhibition in rat paw edema. Thus findings suggest microemulsion based transdermal system as promising formulation for dermal delivery of dexamethasone.

PRONIOSOME BASED TRANSDERMAL DRUG DELIVERY SYSTEM OF PIROXICAM

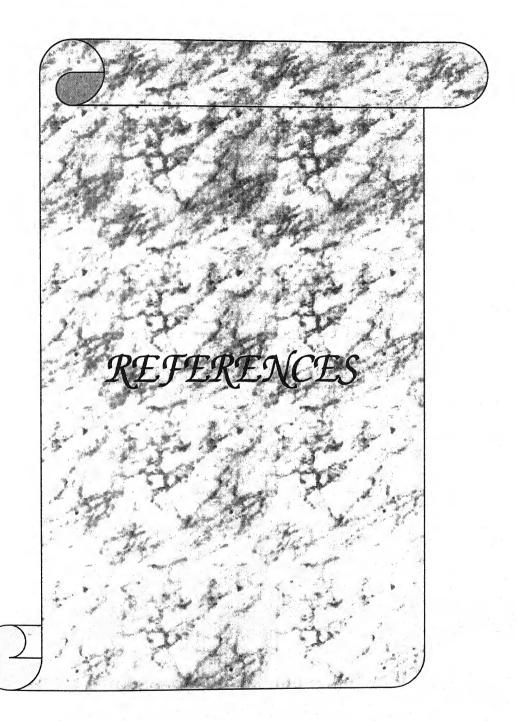
Piroxicam, is a widely used non-steroidal anti-inflammatory drug, its low dose makes it a potential drug of choice for dermal delivery moreover dermal delivery has its advantages. A potential way to make drug permeate is via

lipid vesicles but they too are associated with their drawbacks. The study thus investigates the potential of these provesicular systems to carry drug through the skin. Proniosomes of piroxicam were prepared using various non ionic surfactants, Span 20, Span 40, Span 60 and Span 80 with cholesterol. The formulations were evaluated for surface morphology by scanning electron microscopy, particle size, entrapment efficiency, in-vitro drug permeation, skin irritation, stability and anti-inflammatory potential. It was observed that Span 60 produced vesicles of smallest size and higher entrapment efficiency. The entrapment efficiency was further enhanced by incorporation of egg lecithin. Niosomes of Span 60 and lecithin were able to deliver the drug much efficiently and were chosen for further optimization. Proniosomes were prepared by conventional technique and on maltodextrin and sorbitol as base. Highest flux achieved was 16.33 µg/cm²/h, an enhancement of 3.78 times as compared to control gel from proniosome based Carbopol hydrogel prepared by conventional technique. The anti-inflammatory studies confirmed the enhancement potential of vesicular system for transdermal delivery of piroxicam.

COMPARATIVE STUDY

The stability studies revealed that formulations were stable in the dosage form designed. Model fitting data revealed that formulations were capable of delivering drug at close to zero order rate, however there was strong deviation in case of proniosomes of piroxicam. This deviation requires in depth analysis

and probably could be due to lack of stability of lipid vesicles. The permeation rate across human cadaver skin and shed snake was also studied. Shed snake skin demonstrated least permeability while rat abdominal skin the highest. The study revealed that the drugs under study could be effectively delivered through skin.



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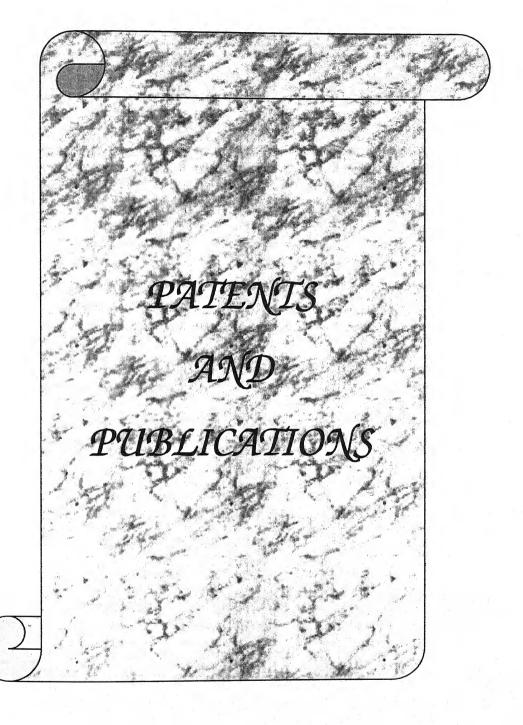
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LIST OF PATENTS

♦ ENHANCED DRUG DELIVERY

(Application no. 273/DEL/2008 filed on 31/01/2008)

MICROEMULSION

(Application no. 274/DEL/2008 filed on 31/01/2008)

♦ TREATMENT OF PAIN AND INFLAMMATION

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LIST OF PUBLICATIONS

- ♦ NSAIDs DERMAL DELIVERY: A REVIEW online on Pharmainfonet.com
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LIST OF ARTICLES ACCEPTED FOR PUBLICATIONS

- MICROEMULSION BASED HYDROGEL FORMULATION OF FOR
 TRANSDERMAL DELIVERY OF DEXAMETHASONE in Asian Journal of
 Pharmaceutics.
- ◆ EFFECT OF ALCOHOLS AND ENHANCERS ON PERMEATION ENHANCEMENT OF KETOROLAC in Asian Journal of Pharmaceutics.



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'54) Title of the invention: ENHANCED DRUG DELIVERY

:A61K31/573	(71)Name of Applicant :
:NA	1)AMRISH CHANDRA
:NA	Address of Applicant :INSTITUTE OF
:NA	PHARMACY, BUNDELKHAND UNIVERSITY,
:NA	KANPUR POAD, JHANSI, U.P., PIN: 284 128,
:NA	INDIA. Uttar Pradesh India
:NA	2)PRAMOD KUMAR SHARMA
:NA	3)RAGHUVEER IRCHHIAYA
:NA	(72)Name of Inventor :
	1)AMRISH CHANDRA (India)
:NA	2)PRAMOD KUMAR SHARMA (India)
:NA	3)RAGUVEER IRCHHIAYA (India)
	:NA :NA :NA :NA :NA :NA :NA

57) Abstract:

An abrasion hydrogel contains in a gel base a therapeutically effective concentration of cetorolac and an abrasive abrading agent, preferably powdered apricot shell powder. The ibrasion hydrogel is applied to areas of the skin that are to be treated and is massaged into the ikin. The scrub is thereafter dusted off with a soft cloth. The abrasion hydrogel contains cetorolac in an amount between about 1 and 10% by weight.

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(71)Name of Applicant:

1)AMRISH CHANDRA

Address of Applicant :INSTITUTE OF PHARMACY, BUNDELKHAND UNIVERSITY, KANPUR POAD, JHANSI, U.P., PIN: 284 128, INDIA. Uttar Pradesh India

2)PRAMOD KUMAR SHARMA

(72)Name of Inventor:

1) AMRISH CHANDRA (India)

2)PRAMOD KUMAR SHARMA (India)

57) Abstract:

lutmeg oil-in-water microemulsion that is transparent or translucent in appearance and comprises nutmeg oil, a surfactant, a cosurfactant or cosolvent and a water phase comprising vater and a theraputically active oil-soluble drug; the oil comprises from 1 to 35 %w/w. The nvention relates generally to a pharmaceutical dosage composition having nutmeg oil and a herapeutically effective amount of a non-steroidal anti-inflammatory drug in a microemulsion hat can be used for the treatment of pain and inflammation by topical or transdermal application. More particularly, the pharmaceutical dosage combination includes nimesulide, liclofenac or dexamethasone or their pharmaceutically active salt in combination with nutmeg sil microemulsion for topical or transdermal administration.

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(54) Title of the invention: TREATMENT OF PAIN AND INFLAMMATION

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(51) International classification	:A61K	(71)Name of Applicant :
(31) Priority Document No	:NA	1)AMRISH CHANDRA
(32) Priority Date	:NA	Address of Applicant :INSTITUTE OF
(33) Name of priority country	:NA	PHARMACY, BUNDELKHAND UNIVERSITY,
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(87) International Publication No	:NA	2)PRAMOD KUMAR SHARMA
[61) Patent of Addition to	:NA	(72)Name of Inventor :
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(62) Divisional to Application	:NA	
Number	:NA	
Filing Date		
57) Abstract :		

57) Abstract :

In exfoliating scrub contains in a microemulsion gel base a therapeutically effective concentration of nonsteriodal anti inflammatory drug (NSAID"s) and an abrasive exfoliating igent, preferably powdered apricot shells. The scrub is applied topically to areas of the skin hat are to be treated and is massaged into the skin. The scrub is thereafter dusted off from the skin with a soft cloth. The scrub contains NSAID"s in an amount between about 1 and 10% by veight.

Number of Pages = 6

NSAIDS Dermal Delivery: A Review

By - 03/05/2007 in <u>Latest Reviews Vol. 5 Issue 2 2007</u>



Amrish Chandra

The dermal absorption of the various compounds can be predicted from physicochemical parameters obtainable from commercially available software and various mathematical models stated by various authors.

If this information is combined with knowledge of the potency of the NSAID, rational judgments can be made about the suitability of the drug for further development. This publication seeks to examine a number of representative NSAIDs with due potential for dermal delivery.

Nonsteroidal anti-inflammatory agents (NSAIDs) class of drugs which budded from the bark of willow in the mid-eighteenth century ¹ has now evolved as selective COX-2 inhibitors. There has been a rapid increase in the number of products that have been designed to deliver NSAIDs. These include simple creams, gels and more complex transdermal systems. A number of approaches have been continuously investigated so as to enhance dermal delivery by use of prodrugs ², ultrasound ³, inotophorosis ⁴, microneeedles ⁵ but the most common and popular means is by the use of chemical penetration enhancers ⁶. The choice of the most appropriate drug depends on a number of factors which includes its potency, its ability to permeate the stratum corneum, its lack of local skin toxicity and stability towards metabolizing enzymes present on the skin surface.

The advent of COX-2 inhibitors, gave much relief to the patients battling the adverse effects of NSAIDs. NSAIDs have a number of side effects associated with them; especially the oral route has a lot many limitations. NSAIDs administered orally causes ulceration and stricture formation in esophagus, stomach and duodenum and may cause severe bleeding, perforation and obstruction ^{7.8}, renal failure ^{9,10} and congestive cardiac failure ^{11,12,13} and cancer is also sometimes associated with their use ^{14,15,16}.

With the discovery of selective COX-2 inhibitors the side effects due to non selective cox inhibitors were overcome and therefore they became the drug of choice. Rofecoxib was marketed as the non-steroidal anti-inflammatory drug of choice because selective inhibition of enzyme made it highly effective but free from gastrointestinal toxicity. But then coxibs too were not devoid of adverse effects. Their withdrawal started in September

2004 with rofecoxib (Vioxx), a product of Merck after the adenomatous polyp prevention trial ¹⁷ showed an increase in major cardiovascular events in patients with a history of colorectal adenomas who were randomized to received Vioxx, compared with those on placebo group. The adverse effect was certainly not limited to rofecoxib and other coxibs like valdecoxib (Bextra, Pfizer) were also withdrawn. It was seen that valdecoxib taken after coronary artery bypass grafting was associated with an increased incidence of cardiovascular events ¹⁸; and the adenoma prevention trial with with celecoxib (Celebrex, Pfizer) ¹⁹ also reported an increased risk of cardiovascular events though it was known to be less selective for COX 2 than rofecoxib or valdecoxib. ²⁰ A small increase in the risk of myocardial infarction was also observed for the highly selective lumiracoxib (Prexige, Novartis). ²¹

There has been thus a renewed interest in traditional NSAIDs; but the presence of oral adverse effects necessitates the need for investigating other routes of drug delivery. The dermal absorption of the various compounds can be predicted from physicochemical parameters obtainable from commercially available software and various mathematical models stated by various authors. If this information is combined with knowledge of the potency of the NSAID, rational judgments can be made about the suitability of the drug for further development. This publication seeks to examine a number of representative NSAIDs with due potential for dermal delivery.

Dermal Delivery Of Nsaids

In vitro studies and theoretical considerations indicate that NSAIDs could be effective when applied dermally. Formulation is crucial to good skin penetration. For NSAIDs to be effective, they have to at least penetrate the skin. Only when the drug has entered the lower layers of the skin can it be absorbed by blood and transported to the site of action, or penetrate deeper into areas where inflammation occurs. This can be judged by in vivo and in vitro studies. Though results obtained from in vivo studies are most reliable they have their own limitations. In vitro experiments are conducted in a manner similar to the permeation across skin. A device with two chambers is used; in between them is either an artificial membrane or a piece of skin human or animal. Drug is placed in the donor compartment i.e. on the dorsal surface of the skin. Samples are removed at defined intervals from the receptor chamber, and drug concentrations measured. Drug moves from donor to receptor compartment. It is important that sink conditions are always maintained in the receptor compartment. Depending upon the properties of drug, formulation ingredients and the barrier used, the drug diffuses across.

Table 1: Selected experimental results on NSAID permeation

Sr. No.	DRUG	TYPE	ENH	IANCER	PURPOSE	REFRENCE
			PG,	EtOH,	Inhibition of platele aggregation, reduce Ginside effects	

heilan			hydrocarbon gel		Product of Street, and
١.	Aspirin	Hydrogel	-		22
		Prodrug		Higher flux was achieved	23
2.	Acetaminophen		Glyceryl oleate PEG 400 and EtOH	Transdermal delivery for paediatric use	1
3.	Diclofenac		Olesan oil, DMSO	Penetration enhancement, significant anti- inflammatory activity	25
		Spray gel		1	
			Phonophorosis Radiofrequency-	Enhanced drug permeation Flux of 23.0	27
		type TD		mcg/sq.cm/h was achieved.	28
		Emulgel	Microemulsion	Eight fold increase in plasma drug level	29
	D:0 : 1		Polyamidoamine dendrimer	Enhanced permeation	20
1.	Diflunisal	Dendrimer Reservoir type TD patch		Increased permeation, significant increase in anti-inflammatory activity	Y
5.	Flurbiprofen	Iontophorosis	Lemon oil, HPMC gel	Rapid permeation	31
					32
		Patch	PG, eudragit E, RL	Reduced crystallization	

6.	Ibuprofen			The delicates to the contract to the property ones are about 200 as a contract of the delicate and design and in	33
		Gel		In vivo enhancement due to vasodilatory effect of menthol	ł
		Prodrug	Thiolated derivative	Better permeation and hydrolysis of prodrug in plasma	
7.	Indomethacin	Ointment			36, 37
		Ploxamer gel	Cubisome	Prolonged release	38
		Nanocapsules	cyanoacrylate	Small particle size lead to enhanced permeation	ł .
	nder o trap o dominante medi est	Liposomes	-	Prolonged anti- inflammatory activity	40
		Reservoir type TD system	Cream	Controlled release was achieved	41
		Hydrogel	Nanostructured lipid carrier	Prolonged anti- inflammatory activity	42
8.	Ketoprofen	Gel	Ultrasound	Higher local drug concentration	43
		Gel	Limonene	Enhanced permeation	44
9.	Ketorolac	Prodrug		Log P was increased from 1.04 to 4.28	45
•		Gel	prodrug and	Better enhancement was obtained from prodrug	
		Niosomes	Fatty alcohols as bilipid stabilizers		47
•		Prodrug	Ester derivative	Optimum lipophilicity and improvedtransdermal delivery	48
		TD liquid reservoir system	microporous	Plasma concentration of 0.82 mcg/ml was achieved	
		Ultrasound		Enhanced permeation	50

	and in the state of the state o	Iontophorosis	enhancement with chemical enhancer and ultrasound	independent of passive diffusion and maximum permeation was achieved when all three were used.	
Carlo M. Propinsky Co. Carlo		Proniosomes		Improved permeation and reduced lag time	53
10.	Naproxen	Patch	Eudragit E–100	Dug release followed Higuchi model	54
11.	Piroxicam	Poloxamer gel	Nonionic surfactant	Enhanced permeation	55
		Iontophorosis		Significant increase in amount of drug delivered into SC	1
1		Organogel		Enhanced oedema inhibition	57
		Gel	Poloxamer 407	Significant inhibition of carragenin induced rat paw edema	
12.	Nimisulide	Carbopol gel	Nanocapsules, nanoemulsion	Characterization studies	59
		Niosomes	In carbopol gel	Five fold increase in mean percentage edema inhibition	

Conclusion

It is evident from these and other studies various features of drug penetration:

- 1. Importance of the drug: Theoretical and experimental results suggest that a balance between lipid and aqueous solubility is needed to optimise penetration. Use of prodrug has led to enhancement of permeability ⁶¹.
- 2. Importance of the formulations: Formulations can make a huge difference in drug permeation. Creams are generally less effective than gels or sprays, but newer formulations like microemulsions have greater potential. Reservoir type systems are effective in giving zero order release rate.
- 3. Use of physical enhancement (iontophorosis, ultrasound, microneedles) have greater potential for drug delivery and high molecular weight proteins like insulin can also be delivered via skin by their use 62 .

4. NSAID's dermal use is has been questioned because they cost more than generic oral NSAIDs. But oral NSAIDs are associated with significant adverse events (gastrointestinal, heart failure, and renal failure). Topical NSAIDs have much lower plasma concentrations, and are not associated with higher rates of adverse events, or at any rate of gastrointestinal adverse events. Moreover they are easy to apply and easier for patient to comply.

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About Authors:



Chandra A

Rajiv Academy for Pharmacy, NH #2 Delhi-Mathura Bypass Road, Mathura U.P., India - 286001

e-mail: amrish chandra@yahoo.com.

Mobile :91-94128-95677



Dr.P.K.Sharma

M.Pharm, Ph.D, Principal, KIET School of Pharmacy, Ghaziabad-Meerut Road, Ghaziabad, U.P. India

Microemulsions: An Overview

By - 03/11/2008 in <u>Latest Reviews Vol. 6 Issue 2 2008</u>



Mr. Amrish Chandra

Microemulsions are a thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules.

A microemulsion is considered to be a thermodynamically or kinetically stable liquid dispersion of an oil phase and a water phase, in combination with a surfactant. The dispersed phase typically comprises small particles or droplets, with a size range of 5 nm-200 nm, and has very low oil/water interfacial tension.

Introduction

The term "microemulsion" refers to a thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules. A microemulsion is considered to be a thermodynamically or kinetically stable liquid dispersion of an oil phase and a water phase, in combination with a surfactant. The dispersed phase typically comprises small particles or droplets, with a size range of 5 nm-200 nm, and has very low oil/water interfacial tension. Because the droplet size is less than 25% of the wavelength of visible light, microemulsions are transparent. The microemulsion is formed readily and sometimes spontaneously, generally without high-energy input. In many cases a cosurfactant or cosolvent is used in addition to the surfactant, the oil phase and the water phase.

Three types of microemulsions are most likely to be formed depending on the composition:

· Oil in water microemulsions wherein oil droplets are dispersed in the continuos aqueous phase

- · Water in oil microemulsions wherein water droplets are dispersed in the continuous oil phase;
- · Bi-continuous microemulsions wherein microdomains of oil and water are interdispersed within the system.

In all three types of microemulsions, the interface is stabilized by an appropriate combination of surfactants and/or co-surfactants.

The key difference between emulsions and microemulsions are that the former, whilst they may exhibit excellent kinetic stability, are fundamentally thermodynamically unstable and will eventually phase separate ¹. Another important difference concerns their appearance; emulsions are cloudy while microemulsions are clear or translucent. In addition, there are distinct differences in their method of preparation, since emulsions require a large input of energy while microemulsions do not. The latter point has obvious implications when considering the relative cost of commercial production of the two types of system.

Microemulsion formation and stability can be explained on the basis of a simplified thermodynamic rationalization. The free energy of microemulsion formation can be considered to depend on the extent to which surfactant lowers the surface tension of the oil—water interface and the change in entropy of the system such that,

$$DG_f = \gamma DA - TDS$$

where DG_f is the free energy of formation, γ is the surface tension of the oil-water interface, DA is the change in interfacial area on microemulsification, DS is the change in entropy of the system which is effectively the dispersion entropy, and T is the temperature. It should be noted that when a microemulsion is formed the change in DA is very large due to the large number of very small droplets formed. It is must however be recognized that while the value of γ is positive at all times, it is very small (of the order of fractions of mN/m), and is offset by the entropic component. The dominant favourable entropic contribution is the very large dispersion entropy arising from the mixing of one phase in the other in the form of large numbers of small droplets. However, favourable entropic contributions also arise from other dynamic processes such as surfactant diffusion in the interfacial layer and monomer-micelle surfactant exchange. Thus a negative free energy of formation is achieved when large reductions in surface tension are accompanied by significant favourable entropic change. In such cases, microemulsification is spontaneous and the resulting dispersion is thermodynamically stable.

Though it has been know that several factors determine whether a w/o or o/w system will be formed but in general it could be summised that the most likely microemulsion would be that in which the phase with the smaller volume fraction forms the droplets i.e. internal phase.

The surfactants used to stabilise such systems may be:

- (i) Non-ionic
- (ii) Zwitterionic
- (iii) Cationic
- (iv) Anionic surfactants

Various pharmaceutically acceptable excipients available that can be used in microemulsion formulation are:

Long chain or high molecular weight (>1000) surfactants include:

Gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, microcrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidene (PVP).

The low molecular weight (<1000) surfactants include:

Stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, and sorbitan esters.

In microemulsions, one can design the interface of such nanometer sized droplets so that droplet stability and lifespan in humans can be made to last from a few milliseconds to minutes, or even to hours. The interfacial rigidity of the microemulsion droplets plays a key role in the flux of the drugs from such droplets to the cells and tissues. Tailoring of microemulsion systems to control the flux of the drugs can be done so as to customize drug delivery according to individual patient requirements or to specific pharmaceutical needs.

It can be seen that there is a real and continuing need for the development of new and effective drug delivery systems for water insoluble or sparingly soluble drugs. One such approach might be pharmaceutical microemulsions. However, materials must be chosen that are biocompatible, non-toxic, clinically acceptable, and use emulsifiers in an appropriate concentration range, and form stable microemulsions. Thus the formulation developed must be a safe and effective pharmaceutical microemulsion delivery systems.

Advantages Of Microemulsion Over Other Dosage Forms

- · Increase the rate of absorption
- · Eliminates variability in absorption
- · Helps solublize lipophilic drug
- · Provides a aqueous dosage form for water insoluble drugs
- · Increases bioavailability
- · Various routes like tropical, oral and intravenous can be used to deliver the product
- · Rapid and efficient penetration of the drug moiety
- · Helpful in taste masking
- · Provides protection from hydrolysis and oxidation as drug in oil phase in O/W microemulsion is not exposed to attack by water and air.
- · Liquid dosage form increases patient compliance.
- · Less amount of energy requirement.

Review

Microemulsions are colloidal dispersions composed of an oil phase, aqueous phase, surfactant and cosurfactant at appropriate ratios. Unlike coarse emulsions micronized with external energy microemulsions are based on low interfacial tension. This is achieved by adding a cosurfactant, which leads to spontaneous formation of a thermodynamically stable microemulsion. The droplet size in the dispersed phase is very small, usually below 140 nm in diameter, which makes the microemulsions transparent liquids². In principle, microemulsions can be used to deliver drugs to the patients via several routes, but the topical application of microemulsions has gained increasing interest. The three main factors determining the transdermal permeation of drugs are the mobility of drug in the vehicle, release of drug from the vehicle, and permeation of drug into the skin. These factors affect either the thermodynamic activity that drives the drug into the skin or the permeability of drug in the skin, particularly stratum comeum. Microemulsions improve the transdermal delivery of several drugs over the conventional topical preparations such as emulsions 3, 4 and gels 5, 6. Mobility of drugs in microemulsions is more facile 4, 6, 7, as compared to the microemulsion with gel former which will increase its viscosity and further decrease the permeation in the skin ⁵. The superior transdermal flux from microemulsions has been shown to be mainly due to their high solubilization potential for lipophilic and hydrophilic drugs. This generates an increased thermodynamic activity towards the skin ^{4, 7, 8}. Microemulsions may affect the permeability of drug in the skin. In this case, the components of microemulsions serve as permeation enhancers. Several compounds used in microemulsions have been reported to

improve the transdermal permeation by altering the structure of the stratum corneum. For example, short chain alkanols are widely used as permeation enhancers ^{9, 10, 11}. It is known that oleic acid, a fatty acid with one double bond in the chain structure, perturbs the lipid barrier in the stratum corneum by forming separate domains which interfere with the continuity of the multilamellar stratum corneum and may induce highly permeable pathways in the stratum corneum ^{12, 13, 14}. Isopropyl myristate (IPM) is used as a permeation enhancer in transdermal formulations, but the mechanism of its action is poorly understood ¹⁵. Nonionic surfactants are widely used in topical formulations as solubilizing agents but some recent results indicate that they may affect also the skin barrier function ¹⁶. It is of interest to explore the effects of these components in the organized microemulsion structures. The aim of the present study was to investigate the potential of several microemulsion formulations in transdermal delivery of lipophilic drugs.

A unique attempt was made ¹⁷ to emulsify coconut oil with the help of polyoxyethylene 2-cetyl ether (Brij 52) and isopropanol or ethanol, forming stable isotropic dispersion thus paving way for use of plant and vegetable oil to be used as oil phase in microemulsion.

The surfactants used to stabilise such systems may be:

(i) Non-ionic, (ii) Zwitterionic, (iii) Cationic and (iv) Anionic surfactants

A combinations of these, particularly ionic and non-ionic, can be very effective at increasing the extent of the microemulsion region. Examples of non-ionics include polyoxyethylene surfactants such as Brij 35 (C₁₂E₃₅) or a sugar esters such as sorbitan monooleate (Span 80). Phospholipids are a notable example of zwitterionic surfactants and exhibit excellent biocompatibility. Lecithin preparations from a variety of sources including sovbean and egg are available commercially and diacylphosphatidylcholine as its major constituent 18, 19, 20, 21. Quaternary ammonium alkyl salts form one of the best known classes of cationic surfactants, with hexadecyltrimethyl ammonium bromide (CTAB) (Rees et al., 1995), and the twin-tailed surfactant didodcecylammonium bromide (DDAB) are amongst the most well known (Olla et al., 1999). The most widely studied anionic surfactant is probably sodium bis-2ethylhexylsulphosuccinate (AOT) which is twin-tailed and is a particularly effective stabiliser of w/o microemulsions 22.

Attempts have been made to rationalise surfactant behaviour in terms of the hydrophile-lipophile balance (HLB) ²³, as well as the critical packing parameter (CPP) ^{24, 25}. Both approaches are fairly empirical but can be a useful guide to surfactant selection. The HLB takes into account the relative contribution of hydrophilic and hydrophobic fragments of the surfactant molecule. It is generally accepted that low HLB (3–6) surfactants are favoured for the formation of w/o microemulsions whereas surfactants with high HLBs (8–18) are preferred for the formation of o/w microemulsion systems. Ionic surfactants such as sodium dodecyl sulphate which have HLBs greater than 20, often require the presence of a cosurfactant to reduce their effective HLB to a value within the range

required for microemulsion formation. In contrast, the CPP relates the ability of surfactant to form particular aggregates to the geometry of the molecule itself.

In most cases, single-chain surfactants alone are unable to reduce the oil /water interfacial tension sufficiently to enable a microemulsion to form, a point made in a number of pertinent microemulsions reviews ^{26, 27, 28, 29, 30}. Medium chain length alcohols which are commonly added as cosurfactants, have the effect of further reducing the interfacial tension, whilst increasing the fluidity of the interface thereby increasing the entropy of the system ^{27, 28}. Medium chain length alcohols also increase the mobility of the hydrocarbon tail and also allow greater penetration of the oil into this region.

Preparation Of Microemulsion

The drug is be dissolved in the lipophilic part of the microemulsion i.e. Oil and the water phases can be combined with surfactant and a cosurfactant is then added at slow rate with gradual stirring until the system is transparent. The amount of surfactant and cosurfactant to be added and the percent of oil phase that can be incorporated shall be determined with the help of pseudo-ternary phase diagram. Ultrasonicator can finally be used so to achieve the desired size range for dispersed globules. It is then be allowed to equilibrate.

Gel may be prepared by adding a gelling agent to the above microemulsion. Carbomers (crosslinked polyacrylic acid polymers) are the most widely used gelling agent.

Construction Of Phase Diagram

Pseudo-ternary phase diagrams of oil, water, and co-surfactant/surfactants mixtures are constructed at fixed cosurfactant/surfactant weight ratios. Phase diagrams are obtained by mixing of the ingredients, which shall be pre-weighed into glass vials and titrated with water and stirred well at room temperature. Formation of monophasic/ biphasic system is confirmed by visual inspection. In case turbidity appears followed by a phase separation, the samples shall be considered as biphasic. In case monophasic, clear and transparent mixtures are visualized after stirring, the samples shall be marked as points in the phase diagram. The area covered by these points is considered as the microemulsion region of existence.

Characterization Of Microemulsion

The droplet size, viscosity, density, turbidity, refractive index, phase separation and pH measurements shall be performed to characterize the microemulsion.

The droplet size distribution of microemulsion vesicles can be determined by either light scattering technique or electron microscopy. This technique has been advocated as the best method for predicting microemulsion stability.

· Dynamic light-scattering measurements.

The DLS measurements are taken at 90° in a dynamic light-scattering spectrophotometer which uses a neon laser of wavelength 632 nm. The data processing is done in the built-in computer with the instrument.

· Polydispersity

Studied using Abbe refractometer.

· Phase analysis

To determine the type if microemulsion that has formed the phase system (o/w or w/o) of the microemulsions is determined by measuring the electrical conductivity using a conductometer.

· Viscosity measurement

The viscosity of microemulsions of several compositions can be measured at different shear rates at different temperatures using Brookfield type rotary viscometer. The sample room of the instrument must be maintained at 37 ± 0.2 °C by a thermobath, and the samples for the measurement are to be immersed in it before testing.

In Vitro Drug Permeation Studies

· Determination of permeability coefficient and flux

Excised human cadaver skin from the abdomen can be obtained from dead who have undergone postmortem not more than 5 days ago in the hospital. The skin is stored at 4C and the epidermis separated. The skin is first immersed in purified water at 60C for 2 min and the epidermis then peeled off. Dried skin samples can be kept at -20C for later use.

Alternatively the full thickness dorsal skin of male hairless mice may be used. The skin shall be excised, washed with normal saline and used.

The passive permeability of lipophilic drug through the skin is investigated using Franz diffusion cells with known effective diffusional area. The hydrated skin samples are used. The receiver compartment may contain a complexing agent like cyclodextrin in the receiver phase, which shall increase the solubility and allows the maintenance of sink conditions in the experiments. Samples are withdrawn at regular interval and analyzed for amount of drug released.

In Vivo Studies

· Bioavailability studies: Skin bioavailability of topical applied microemulsion on rats

Male Sprague—Dawley rats (400–500 g), need to be anesthetized (15 mg/kg pentobarbital sodium i.p.) and placed on their back. The hair on abdominal skin shall be trimmed off and then bathed gently with distilled water. Anesthesia should be maintained with 0.1-ml pentobarbital (15 mg/ml) along the experiment. Microemulsions must be applied on the skin surface (1.8 cm²) and glued to the skin by a silicon rubber. After 10, 30 and 60 min of in vivo study, the rats shall be killed by aspiration of ethyl ether. The drug exposed skin areas shall be swabbed three to four times with three layers of gauze pads, then bathed for 30 s with running water, wiped carefully, tape-stripped (X10 strips) and harvested from the animals.

· Determination of residual drug remaining in the skin on tropical administration.

The skin in the above permeation studies can be used to determine the amount of drug in the skin. The skin cleaned with gauze soaked in 0.05% solution of sodium lauryl sulfate and shall bathed with distilled water. The permeation area shall be cut and weighed and drug content can be determined in the clear solution obtained after extracting with a suitable solvent and centrifuging.

Pharmacological Studies

Therapeutic effectiveness can be evaluated for the specific pharmacological action that the drug purports to show as per stated guidelines.

Estimation Of Skin Irritancy

As the formulation is intended for dermal application skin irritancy should be tested. The dorsal area of the trunk is shaved with clippers 24 hours before the experiment. The skin shall be scarred with a lancet. 0.5 ml of product is applied and then covered with gauze and a polyethylene film and fixed with hypoallergenic adhesive bandage. The test be removed after 24 hours and the exposed skin is graded for formation of edema and erythema. Scoring is repeated a 72 hours later. Based on the scoring the formulation shall be graded as 'non-irritant', 'irritant' and 'highly irritant'.

Stability Studies

The physical stability of the microemulsion must be determined under different storage conditions (4, 25 and 40 °C) during 12 months.

Fresh preparations as well as those that have been kept under various stress conditions for extended period of time is subjected to droplet size distribution analysis. Effect of surfactant and their concentration on size of droplet is also be studied.

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About Authors:



Mr. Amrish Chandra

Asst. Prof., Ishwarchand Vidhya Sagar Institute of Pharmacy, Mathura, U.P. India

e-mail: amrish chandra@yahoo.com. Mobile:91-94128-95677

Author for correspondence



Dr.P.K.Sharma

M.Pharm, Ph.D, Principal, KIET School of Pharmacy, Ghaziabad-Meerut Road, Ghaziabad 201 206, Uttar Pradesh

-Notes-

Transdermal Delivery of Ketorolac

Chandra Amrish*,a and Sharma Pramod Kumarb

^aInstitute of Pharmacy, Bundelkhand University, Kanpur Road, Jhansi 284 128 (U.P.), India, and ^bR.V. Northland Institute, 18 Km Gaziabad-Bulandshahar Road, G.T. Road (Greater Noida Phase II), Dadri, Gautam Budh Nagar 203 207 (U.P.), India

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A reservoir type transdermal patch for delivery of ketorolac, a potent analgesic agent was studied. The low permeability of skin is the rate-limiting step for delivery of most of the drugs. Studies were carried out to investigate the effect of permeation enhancers on the *in vitro* permeation of ketorolac across rat skin. The reservoir type transdermal patch was fabricated and the core was filled with gel system of a non ionic polymer HPMC (hydroxypropyl methyl cellulose) formulated in PBS (phosphate buffer saline) solution of pH of 5.4 along with isopropyl alcohol at 25% w/w concentration. Various permeation enhancers' viz. dimethyl sulphoxide, d-limonene, eucalyptus oil and transcutol (diethylene glycol monoethyl ether) were incorporated into the gel system. Permeation enhancement of ketorolac with different enhancers followed the order eucalyptus oil> transcutol> DMSO> d-limonene. Cyclic terpene containing eucalyptus oil was found to be the most promising chemical permeation enhancer for transdermal delivery of ketorolac. The increase in concentration of eucalyptus oil further enhanced drug permeation with maximum flux being achieved at 10% w/w of $66.38 \, \mu g/cm^2/h$. Further enhancement of permeation rate of ketorolac across skin was attained by application of abrading gel containing crushed apricot seed onto the skin. There was 5.16 times enhancement and flux of $93.10 \, \mu g/cm^2/h$ was attained. A reservoir type transdermal patch for delivery of ketorolac thus appears to be feasible of delivering ketorolac across skin.

Key words—ketorolac; skin; penetration enhancer; pretreatment; abrasive

INTRODUCTION

Ketorolac ((\pm)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, 2-amino-2- (hydroxy methyl)-1,3-propanediol; Mol. wt. 376.4 g/mol) is a prostaglandin synthetase inhibitor, a nonsteroidal anti-inflammatory drug with potent analgesic and moderate anti-inflammatory activities.1) Ketorolac has been studied clinically and its efficacy and safety as an analgesic in cancer and postoperative pain has been established. Administered as injectable and oral formulations, it has shown high analgesic potency almost equivalent to that of morphine.2) Unlike narcotic analgesics, ketorolac does not alter gastric motility or adversely affect respiration, nor is it associated with addiction potential as in case of narcotic analgesics; therefore, ketorolac is a relatively more favorable therapeutic agent for the management of moderate to severe pain.3)

Thus developing an alternative dosage form that is easy to administer; is painless; non invasive; easy to comply and avoids first pass metabolism, is worth-

while. The transdermal route encompasses all the above advantages.

Despite these advantages, only a limited number of drugs can be administered percutaneously, due to low skin permeability of most drugs across the skin. The penetration through stratum corneum is the rate-limiting step for delivery of most of the drugs. To overcome this problem, vehicles, 4) penetration enhancers, 5) ultra sound 6) and electro-transport 7) facilitated transdermal systems have been attempted in the development of a transdermal delivery system of ketorolac. Prodrug approach has also been investigated for enhanced dermal delivery. 8,9) However, the most widely used technique involves use of chemical penetration enhancers or solvents that modify the thermodynamic activity.

In the present study, we therefore investigate the effects of penetration enhancers on the *in vitro* permeation of ketorolac from hyrogel gel formulation across rat abdominal skin to examine the feasibility of developing a transdermal system.

EXPERIMENTAL

Materials Ketorolac was obtained as a gift sam-

ple from Ranbaxy Laboratories, Devas, India. Hydroxy propyl methylcellulose, HPMC (Methocel® K₁₅ M) was gifted by Colorcon Asia Pvt. Ltd., Goa, Isopropyl Alcohol (IPA), dimethyl sulphoxide (DMSO) and Eucalyptus oil were purchased from Central Drug House, New Delhi and comprised 80-85 % w/w cineol. d-limonene was obtained from Hi Media Labs, Mumbai. Transcutol® P (diethylene glycol monoethyl ether) was obtained from Gattefosse, France through Colorcon Asia Pvt. Ltd., Goa. Commercially available adhesive tape was procured from 3M (TM) Milipore (TM) tape (1530-3) was used. The adhesive used was Acrylate (25-35% w/w) and EVA copolymer (0.5-2% w/w) and the moisture vapor transmission rate was 4200 g/m²/24 h. Other chemical and reagents used were of analytical grade. The experimental protocol was approved by the institutional animal ethical committee, Institute of Pharmacy, Bundelkhand University, Jhansi. The reported experiments were carried out in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), constituted under the provision of Section 15 of the Prevention of Cruelty to Animals Act, 1960 of the Indian constitution.

Preparation of Ketorolac Gel System and Fabrication of Reservoir Type Patch Ketorolac gel system was prepared in PBS (phosphate buffer saline) solution by dissolving ketorolac and adding HPMC (hydroxyl propyl methylcellulose) with continuous stirring so as to uniformly disperse the polymer. Permeation enhancers were mixed with the vehicle before adding the polymer. The gel was kept overnight at ambient temperature in a tightly closed container to allow uniform gelling (cold dispersion method).

Transdermal patches (reservoir type) of ketorolac were fabricated by filling ketorolac gel preparation (0.25 g per cm sq.) within a shallow compartment made of a hollow ring shaped device and drug impermeable backing membrane (laminated aluminum foil). A micro porous adhesive tape of a larger area was stuck onto the impermeable backing membrane to bring the transdermal patch in intimate contact with the skin. The device was closed by a release liner on the open side (Fig. 1).

Preparation and Method of Application of Abrasive Gel

The abrasive gel was prepared by heating distilled water to 80°C and adding to it crushed apricot seed (5% w/w) (Prunus armeniaca). The

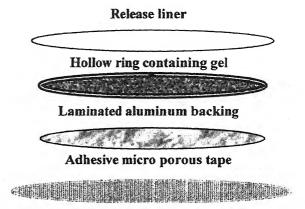


Fig. 1. Design of Fabricated Reservoir Type Patch for Transdermal Administration of Ketorolac

size of crushed pieces ranged between 354 and 251 μ m. The polymer HPMC (2% w/w) was then added slowly and allowed to disperse uniformly (hot dispersion method). It was then cooled to form viscous gel into which was entrapped crushed apricot shell pieces.

One g of the gel was topically applied with the help of index finger on the depilated site before application of formulation and was gently massaged onto the skin for a period of two minutes and pressure ranging between 30-50 g/cm². The crushed apricot pieces were dusted off with soft cloth.

In Vitro Skin Permeation Studies The abdominal skin of Sprague-Dawley rat was clamped between the donor and the receptor chamber with the stratum corneum surface facing the receptor compartment of modified Keshary Chien diffusion cell after removing the hair with a clipper. The effective diffusion area of the cell was 2.0 cm² and had a receptor volume of 11 ml. The receptor chamber was filled with freshly prepared PBS (phosphate buffer saline) solution pH 7.4. The diffusion bath was maintained at $37 \pm 1^{\circ}$ C and the solution in the receptor chambers was stirred continuously with the help of magnetic bead. Ketorolac gel (0.5 g) was gently placed in the donor chamber and spread evenly on the rat abdominal skin. The donor compartment was covered with Parafilm Me to prevent evaporative loss. One ml of the solution in the acceptor chamber was removed for drug content determination and replaced immediately with an equal volume of fresh PBS solution pH 7.4. Drug concentration was determined UV spectrophotometrically.

Analysis of In Vitro Drug Release Study Samples Samples withdrawn were filtered through Whatmann filter paper No. 42 and diluted whenever necessary. The samples were analyzed spectrophotometrically at 324 nm. The concentration of drug was determined from the regression equation generated from the calibration curve, y=0.0285x+0.0012, $R^2=0.999$.

Effect of Permeation Enhancers Ketorolac gel system consisted of 2% w/w ketorolac and 2% w/w HPMC (hydroxyl propyl methylcellulose) in PBS solution at pH of 5.4 along with IPA (isopropyl alcohol) at 25% w/w and permeation enhancers. The gel system for the reservoir compartment of the transdermal patch was prepared by cold dispersion method. Various enhancers' viz. DMSO (dimethy sulfoxides), eucalyptus oil, d-limonene and transcutol (diethylene glycol monoethyl ether) were added at 5% w/w concentration. The concentration of the screened enhancer was further optimized at 7.5 and 10% w/w.

Skin Irritation Studies Transdermal patch of ketorolac gel was applied onto the dorsal skin of Wistar rats (220–250 g) which was shaved 24 h prior to study. The site of application was occluded with gauze and covered with non-sensitizing adhesive microporous tape. After 24 h, the gel was removed and the score of erythema was determined by Drazie test¹⁰⁾ as follows: 0- no eryhtema; 1- very slight erythema (barely perceptible); 2- well-defined erythema; 3- moderate to severe eryhtema and 4- severe erythema (beet redness) to slight eschar formation (injuries in depth).

Carrageenan Induced Paw Edema The anti-inflammatory efficacy was evaluated by carrageenan induced paw inflammation in Wistar rat (approx. 220-250 g). The rats were assigned to treatment groups so that each group was weight balanced. Ketorolac was administered via oral (2 mg/kg) and transdermal route 1 h before carrageenan injection. The transdermal patch was securely adhered over the depilated dorsal abdominal skin (9 cm²). The rats received intraplantar injection of 50 μ l of 0.5% w/v carrageenan suspension into the left hind paw subcutaneously by inserting the needle into the central part of the paw. The paw volume was measured and compared to with that found in animals treated with carrageenan alone. The right hind paw which served as control was treated with physiological saline solution without carrageenan.11) The inflammatory response was determined by measuring the changes in paw volumes with a screw gauge (Mitutoyo, Kanagawa, Japan) at 0, 2, 4, 6, 8 and 10 h after the carrageenan injection.

The degree of paw swelling was calculated as:

Swelling(%) =
$$\frac{Vt - V}{V} \times 100$$

where, Vt is the volume of the carrageenan-treated paw, V is that of the non-treated paw.

Inibition(%) =
$$\frac{Sc - St}{Sc} \times 100$$

where, Sc is the swelling of the control paw, St is that of the test formulation treated paw. The AUC (area under the curve) was determined by trapezoidal method.

Acetic Acid Induced Writhing Effect Ketorolac was administered via oral (2 mg/kg) and transdermal route 1 h before administration of writhing agent. The transdermal patch was securely adhered over the depilated dorsal abdominal skin (9 cm²). The rats received intra peritoneally (i.p.) acetic acid (20 mg/kg, 2 ml/kg) in deionized water. The number of writhes (i.e., abdominal constriction followed by dorsiflexion and extension) occurring during a 15 min period beginning and 15 min after acetic acid administration, was measured. The results are expressed as percentage inhibition in writhes.

Histological Studies Transdermal patch formulations were applied for 24 h on the dorsal surface of excised rat skin mounted on the diffusion cell. The transdermal patch was removed and the skin was wiped off with tissue paper, the skins were fixed in 10 % v/v formalin solution in saline for at least 72 h before further processing it. The skin was sectioned vertically and each section was dehydrated and embedded in paraffin wax. The subdivided tissues were stained with hematoxylin and eosin. The sections were observed under microscope and photographed at 40× magnification. Untreated skin served as control.

Data Analysis The *in vitro* skin flux was determined from Fick's law of diffusion considering the transport of drugs across the skin barrier as a process of passive diffusion. J_{SS} , the skin flux $(\mu g/cm^2/h)$, was determined from the slope of the linear portion of the cumulative amount permeated per unit area *versus* time plot. The lag time (T_{lag}, h) was determined by extrapolating the linear portion of the curve to the abscissa. Enhancement ratio (ER) was calculated from the following equation:

 $ER = J_{ss}$ of test gel/ J_{ss} of control gel

Statistical Analysis The results were analyzed

by paired, two tailed Student's t-test using Statistica for Windows (Version 5.0) from StatSoft, Inc., USA. The results were evaluated at probability level of 0.05. The results are reported as mean ±SD.

RESULTS AND DISCUSSION

Effect of Permeation Enhancers Effect of various chemical permeation enhancers on the permeation of ketorolac from transdermal formulations across rat abdominal skin was investigated. The enhancers evaluated were DMSO, eucalyptus oil, dlimonene and transcutol. The effectiveness of the enhancer was determined by comparing the steady state flux of ketorolac from chemical enhancer containing gel system and that of control gel system. In vitro permeation profile is presented in Fig. 2 and the permeation parameters obtained after data analysis are reported in Table 1. Among the penetration enhancers evaluated the following order was observed; eucalyptus oil> transcutol >DMSO> d-limonene.

DMSO demonstrated a flux of $27.97 \,\mu g/cm^2/h$ and a T_{lag} of 1.97 h. There was an enhancement of 1.55 times. Being a powerful solvent DMSO can mix isothermally with water, it can displace water from the lipid head groups creating gaps around these head groups. DMSO is also capable of displacing water bound to protein head groups. Moreover due to its solvent power, high levels of sulfoxide within the membrane can improve drug partitioning and thus increase the flux. 12

Transcutol showed a flux of $29.72 \,\mu\text{g/cm}^2/\text{h}$ and T_{lag} of 2.05 h with ER of 1.65. Recent studies have shown that transcutol significantly increases the per-

cutaneous penetration of various active substances, particularly when used in combination with suitable cosolvents. 13-17) Transcutol demonstrated enhanced flux in comparison to DMSO but failed to reduce the lag time. This probably could be due to enhanced accumulation of ketorolac in skin. This enhancer has also shown skin accumulation of topically applied compounds without an increase in transdermal permeation. 18)

Essential oils like eucalyptus oil are reported to be effective penetration enhancers for 5-flouorouracil traversing in human skin *in vivo* with maximum enhancement ratio of 34-fold.¹⁹⁾ The principal terpene element within eucalyptus oil is 1, 8-cineole, a cyclic

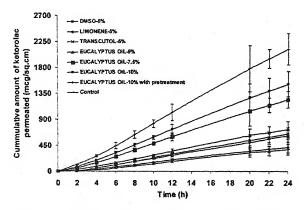


Fig. 2. In Vitro Permeation Profile of Ketorolac through Rat Abdominal Skin from Ketorolac Transdermal Gel System Containing Ketorolac (2% w/w), HPMC (2% w/w), IPA (25% w/w) and Different Enhancers Formulated Using PBS Solution of pH 5.4

Standard deviation values are shown for each data point (n=4) on the graph.

Table 1. Permeation Parameters of Ketorolac through Rat Abdominal Skin from Gel System Containing Ketorolac (2% w/w), HPMC (2% w/w), IPA (25% w/w) Formulated Using PBS Solution of pH 5.4 along with the Chemical Enhancers at Mentioned Concentration (n=4)

Permeation enhancers	ERª	T_{lag}^{b} (h) Mean \pm SD (n=4)	J_{ss}^{c} (μ g/cm ² /h) Mean±SD (n =4)
Control	1.00	2.71 ± 0.13	18.04±0.17
d-limonene (5% w/w)	1.05	1.67 ± 0.55	18.89 ± 0.62
DMSO (5% w/w)	1.55	1.97 ± 0.60	27.97 ± 0.99
Transcutol (5% w/w)	1.65	2.05 ± 0.30	29.72±1.63
Eucalyptus oil (5% w/w)	1.80	1.34 ± 0.10	32.47 ± 0.83
Eucalyptus oil (7.5% w/w)	3.04	1.14 ± 0.34	54.79 ± 3.17
Eucalyptus oil (10% w/w)	3.68	1.08 ± 0.32	66.38±3.12
Eucalyptus oil (10% w/w) after pretreatment	5.16	1.09 ± 0.40	93.10±3.07

[&]quot; ER: Enhancement ratio, b Tlag: lag time, ' Jss: skin permeation rate (flux).

ethers, and proven enhancer20) but its efficacy as an enhancer are mixed. Eucalyptus oil could not enhance the permeation of lipophilic drug like estradiol. Similar results were also reported for the permeation of indomethacin, a lipophilic molecule. The study revealed that oxygen containing terpenes (carvone, 1, 8-cineole) were ineffective21) whereas hydrocarbon terpenes, especially limonene, were effective towards lipophilic drugs. The results of permeation enhancement of ketorolac, a hydrophilic drug also support the above observations towards which oil containing oxygen containing terpenes were effective. The permeation of penetration enhancer itself from the formulation is essential in order to achieve enhanced permeation of drug. The enhancer being lipophilic is believed to readily permeate out of gel into the skin. Eucalyptus oil at 5% produced a flux of 32.47 μ g/cm²/h, an enhancement of 1.80 and a T_{lag} of 1.34 h. eucalyptus oil demonstrated maximum permeation rate shortest T_{lag}. It was therefore appropriate to study the effect of eucalyptus oil concentration on permeation rate of ketorolac. Increasing the concentration of eucalyptus oil to 7.5% and 10% concentration produced a flux of 54.79 and 66.38 μ g/cm²/h, an enhancement of 3.04 and 3.68 times and reduction of T_{lag} to 1.14 and 1.08 h respectively. Further increase in eucalyptus oil concentration was not justified due to skin irritation potential of the oil on prolonged contact with the skin.

These essential oils probably modify the solvent nature of the stratum corneum, improving drug partitioning into the tissue. Terpenes are generally good solvents and permeate skin well,²²⁾ with loss of terpenes, from a formulation there could be an alteration of the thermodynamic activity of the permeant. Terpenes may also modify drug diffusivity through the membrane and bring about a reduction of the lag time for permeation, indicating increase in diffusivity of the drug through the membrane following terpene treatment. X-ray diffraction studies have also indicated that d-limonene and 1,8-cineole disrupt stratum corneum bilayer lipids.²³⁾

Effect of Pretreatment with Abrading Gel Attempts were made to enhance the permeation of ketorolac across skin by abrading the upper skin layer with the help of abrading gel made of crushed apricot seed. There was 5.16 times enhancement in permeation of ketorolac across rat skin from formulation containing ketorolac (2% w/w), HPMC (2% w/w),

IPA (25% w/w) and eucalyptus oil (10% w/w) prepared in PBS solution of pH 5.4. The J_{ss} and T_{lag} of 93.10 μ g/cm²/h and 1.09 h were attained respectively after pretreatment.

Skin Irritation Studies The results of the skin irritation studies based on visual observation score suggest that the formulations were safe to be applied on skin. The scores for d-limonene and Transcutol were between 0-1; while that for eucalyptus oil and DMSO were between 0-2.

Histological Studies The control skin (Fig. 3A) showed intact stratum corneum with no swelling of epidermis. No inflammatory cells were seen. The normal skin stratification was intact. Various chemical enhancers dramatically affected the skin. Transdermal formulation containing d-limonene fluidized the inner lipid layers and mild increase in the number of inflammatory cells was observed but the stratum corneum was found to be almost intact (Fig. 3B). Formulation containing DMSO demonstrated significant fluidization of the inner dermis; the stratum corneum was seen disrupted though inflammatory cells were not observed (Fig. 3C). Transdermal formulation containing eucalyptus oil fluidized the inner dermal lipids; the skin was found swollen with slight increase in the number of inflammatory cells (Fig. 3D). On treatment of skin with formulation containing transcutol it was observed that only the epidermal portion had swollen with slight fluidization in this part while the basic stratification of the skin was almost maintained (Fig. 3E). The effect of abrading the skin with abrading gel followed by treatment with transdermal formulation containing eucalyptus oil was observed on the skin. Stratum corneum was disrupted the skin lipids were vastly fluidized and the skin appeared swollen (Fig. 3F).

Anti-inflammatory Response The formulation showed a prominent increase in activity in the carrageenan induced paw inflammation model. Fig. 4 represents the anti inflammatory activity after oral administration and after transdermal application after treatment with abrading gel.

Ketorolac transdermal patch formulation demonstrated comparable anti-inflammatory potential to orally administered ketorolac. The anti-inflammatory potential was measured in terms of the AUC of graph plotted between difference in paw diameter and time. Compared to the % AUC for untreated paw which was taken as 100%, oral administration showed swell-

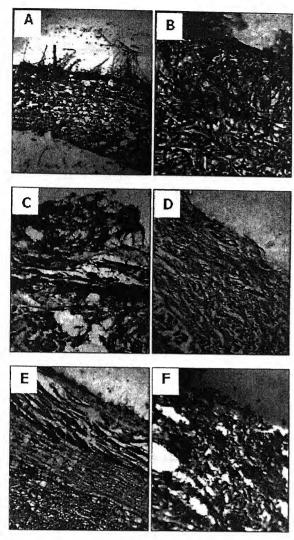


Fig. 3. Histological Findings after Treatment of Rat Abdominal Skin for 24 h with Chemical Permeation Enhancer Gel System Observed under Microscope after Staining with Hematoxylin-eosin Stain

A-Control (10 \times), B-d-limonene (40 \times), C-DMSO (40 \times), D-Eucalyptus oil (40 \times), E-Transcutol (40 \times) and F-Application of eucalyptus oil preparation after pretreatment (40 \times).

ing of 64.04% while transdermal application after pretreatment demonstrated swelling of 60.67%. Maximum percentage inhibition was observed at 6 h and 4 h for oral and transdermal application after pretreatment of 50% and 45.45% respectively. There is clear evidence of enhanced permeation with quicker onset of action for the transdermal formulation applied after pretreatment. Abrasion of skin by crushed seeds of apricot probably helps in removing if not completely at least partially the upper stratum corneum thus aiding permeation enhancement.

Antinociceptive Response There was 88.10%

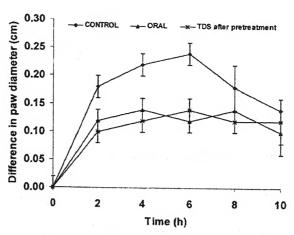


Fig. 4. Anti-inflammatory Activity of Ketorolac on Paw Edema Induced with Carrageenan Injection (0.05 ml of 0.5% w/w) in Rats (Control) and after Oral Administration of Ketorolac Solution (2 mg/kg) (Oral) and on Application of Transdermal Patch of Ketorolac (TDS) after Pretreatment with Abrading Gel

The reservoir transdermal patch formulation contained ketorolac (2% w/w), HPMC (2% w/w), IPA (25% w/w) and eucalyptus oil (10% w/w) formulated using PBS solution of pH 5.4 in the reservoir chamber. Standard deviation values are shown for each data point (n=4) on the graph.

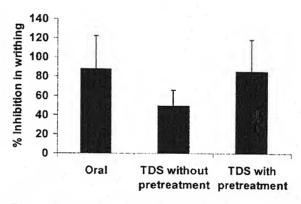


Fig. 5. Antinociceptive Response of Ketorolac 15 min after Induction of Writhes with the Help of Acetic Acid (20 mg/kg; 2 ml/kg) after Oral Administration of Ketorolac (2 mg/kg) and on Application of Transdermal Patch of Ketorolac (TDS) without Pretreatment and after Pretreatment with Abrasive Gel Preparation

The transdermal patch formulation contained ketorolac (2% w/w), HPMC (2% w/w), IPA (25% w/w) and eucalyptus oil (10% w/w) formulated using PBS solution of pH 5.4 in the reservoir chamber. Standard deviation values are shown for each data point (n=4) on the graph.

inhibition in writhing on oral administration was observed while application of transdermal application without pretreatment and after pretreatment demonstrated 50% and 85.71% inhibition in writhing response (Fig. 5). The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be

mediated by peritoneal mast cells,²⁴⁾ acid sensing ion channels²⁵⁾ and the prostaglandin pathways.²⁶⁾ The reservoir type transdermal patch consisting of ketorolac gel thus appears promising in delivering the drug across skin.

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Full Length Research Paper

Proniosome based drug delivery system of piroxicam

A. Chandra* and P. K. Sharma

Institute of Pharmacy, Bundelkhand University, Kanpur Road, Jhansi (U.P.) - 284128, India.

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Piroxicam is a widely used potent non-steroidal anti-inflammatory drug, with due potential for dermal delivery. Permeation of piroxicam from proniosome based reservoir type transdermal gel formulation across excised rat abdominal skin was investigated using Keshery Chein diffusion cell. There was considerable improvement in flux over the control gel formulation. The lipid vesicles were evaluated for entrapment efficiency and vesicle size of niosomes formed. It was observed that Span 60 based formulations produced vesicles of smallest size and higher entrapment efficiency while those of Span 80 produced vesicles of least entrapment efficiency. Incorporation of lecithin further enhanced entrapment efficiency. Proniosomes were prepared by conventional technique and employing maltodextrin and sorbitol as base. The morphology of the proniosomes was studied by scanning electron microscopy. Maximum flux achieved was 35.61 μ g/cm²/h, an enhancement of 7.39 times was achieved for transdermal system based on proniosomal gel as compared to control gel. Anti-inflammatory studies revealed that proniosome based transdermal drug delivery system of piroxicam were promising carriers for delivery of piroxicam. There was significant reduction in carrageenan induced rat paw inflammation compared to control.

Key words: Piroxicam, niosomes, permeation enhancement, dermal delivery.

INTRODUCTION

Colloidal particulate carriers such as liposomes (Mishra et al., 2007) or niosomes (Shahiwala and Misra, 2002) have been widely employed in drug delivery systems and producing them from proniosomes provides them a distinctive advantage. These carriers can act as drug reservoirs and the rate of drug release can be controlled by modification of their composition. These lipid vesicles can carry both hydrophilic drugs (by encapsulation) and hydrophobic drugs (in lipid domain). Due of their capability to carry a variety of drugs, these lipid vesicles have been extensively used in various drug delivery systems (Puglia et al., 2004) like drug targeting (Gupta et al., 2005), controlled release (Barber and Shek, 1993) and permeation enhancement of drugs (Verma et al., 2003). But there remains certain draw backs to be addressed and can be avoided if they are prepared in dry form. Proniosomes, prepared in dry form and hydrated by agitation in hot water to form niosomes provide an alternative with prospective for drug delivery via the

transdermal route (Vora et al., 1998; Hu and Rhodes, 1999).

The transdermal route of drug delivery has many advantages for administration of drugs in local and systemic therapy. But, skin is widely recognized for its effective barrier properties compared with other biological membranes. The low permeability of the skin makes it a minor port of entry for drugs. The vesicular drug delivery is thus potentially beneficial as vesicles tend to fuse and adhere to the cell surface; this is believed to increase the thermodynamic activity gradient of the drug at vesicle-stratum corneum interface thus leading to enhanced permeation rate.

Piroxicam, a non-steroidal anti-inflammatory drug (NSAID), are used in the treatment of dysmenorrheal, various acute and chronic musculoskeletal disorders like rheumatoid arthritis, osteoarthritis etc., and also as potent analgesics (Andersson et al., 1998). However, the use of piroxicam has been associated with a number of gastrointestinal disorders (Schiantarelli and Cadel, 1981). Dermal delivery is an alternative route, but requires a formulation which ensures the deep skin penetration. Several researchers have successfully delivered piroxicam via organogel by Agrawal et al. (2004), buccal

^{*}Corresponding author: E-mail: amrish_chandra@yahoo.com. Tel.: 91-941-2895677.

Table 1. Composition of proniosomal formulations of piroxicam.

Proniosomal code	Span 20 (mg)	Span 40 (mg)	Span 60 (mg)	Span 80 (mg)	Cholesterol (mg)	Lecithin (mg)
S2	360	-	-	-	40	-
S4	-	360	<u>.</u>	-	40	-
S6	-	-	360	-	40	- :
S8		* -	-	360	40	- · -
S6L	-	-	180	-	40	180
S6LM	-	_0 ,-	180	-	40	180
S6LS	- *	•	180	-	40	180

gel by Attia et al. (2004), mucoadhesive system by Cilurzo et al. (2005), microspheres based drug delivery by Raman et al. (2005), Berkland et al. (2004), Georgeta et al. (2004), iontophoresis was attempted by Curdy et al. (2001), cyclodextrin based enhancement was carried out Murthy et al. (2004) and gel based formulation by Shin et al. (2000) and Santoyo et al. (1995).

Thus the study encompasses the ability of lipid vesicles to deliver piroxicam across skin in order to evaluate it transdermal delivery potential. Moreover greater stability can be accorded by proniosomal formulation as compared to niosomes and access their potential towards dermal delivery.

MATERIALS AND METHODS

Piroxicam was procured from Torrent Pharmaceuticals, Ahemdabad, India. Span 20, 40, 60 and 80, chloroform, isopropyl alcohol, maltodextrin, sorbitol, cholesterol, Carbopol 934 was purchased from Central Drug House, New Delhi, India. Dialysis membrane-150 and egg lecithin was purchased from Himedia, Mumbai, India. Due permission was obtained from Institutional Animal Ethics Committee for conduct of animal experimentation (Registration number 716/02/a/CPCSEA)

Preparation of proniosomes

Various proniosomal preparations were formulated using surfactant, cholesterol, lecithin, and piroxicam. The compositions of different proniosomal formulations prepared are listed in Table 1.

Preparation of conventional niosome

Proniosomes were prepared using the method reported (Perrett et al.. 1991) with slight modification. The formulations S2, S4, S6 and S8 prepared comprised of 360 mg of surfactant (Span 20, 40, 60 and 80). 40 mg of cholesterol, and 20 mg of piroxicam in isopropyl alcohol were taken in a wide-mouth glass vial. The open end of the glass vial was covered and the tube was warmed in a water bath at 65°C till the surfactant mixture dissolved completely. However the formulation S6L comprised of 180 mg of Span 60, 40 mg of cholesterol. 180 mg of egg lecithin and 20 mg of piroxicam and was prepared in a similar manner.

Preparation of maltodextrin based proniosome

The formulation S6LM comprised of 180 mg of Span 60, 40 mg of

cholesterol, 180 mg of egg lecithin and 20 mg of piroxicam dissolved in chloroform and iso propyl alcohol mixture (4:1). The mixture was added to a 100 ml round bottom flask containing 0.5 g of maltodextrin powder. The flask was attached to the rotary evaporator (Hicon Grover Enterprises, New Delhi, India) maintained at a temperature of $65\,^{\circ}\mathrm{C}$ using water bath and the flask was rotated at 60 rpm under vacuum until the powder appeared to be dry and free flowing. The dried material (S6LM) was removed from the evaporator and kept under vacuum overnight.

Preparation of sorbitol based proniosomes

0.5 g of sorbitol was placed in a 100 ml round bottom flask attached to a rotary evaporator. Span 60 180 mg, cholesterol 40 mg, lecithin 180 mg and piroxicam 20 mg mixture in chloroform and isopropyl alcohol mixture (4:1) was added slowly on to sorbitol powder bed. Care was taken not to over wet the powder base. The rotary evaporator (Hicon Grover Enterprises, New Delhi, India) was maintained at a temperature of 65 ℃ using water bath and the flask was rotated at 60 rpm under vacuum so as to dry the powder base before further addition of surfactant mixture. The dried material (S6LS) was finally removed and kept under vacuum overnight.

Preparation of piroxicam niosomal gel

Proniosome powder was weighed into screw cap vials to which was added water at 80 °C. The vials were vortex mixed for complete and uniform hydration. The niosomal preparations were then converted into gel by appropriately diluting the proniosomes and adding Carbopol 934 (1%w/w) for ease of handling. The final drug concentration achieved was 0.5%w/w.

Preparation of piroxicam carbopol gel

0.5% w/w piroxicam was dissolved/suspended in saline phosphate buffer pH 7.4 and to it was added carbopol 934 (1% w/v). The gel was finally obtained by addition of triethanolamine.

Fabrication of reservoir type patch of niosomal gel of piroxicam

Transdermal patches (reservoir type) of piroxicam were fabricated by encapsulating niosomal gel preparation of piroxicam within a shallow compartment made of drug impermeable backing membrane (laminated aluminum foil) and a hollow ring shaped compartment. A micro porous tape of a larger area was stuck onto the impermeable backing membrane to bring the transdermal patch in close contact with the skin. The device was closed by a release liner on the open side (Figure 1).

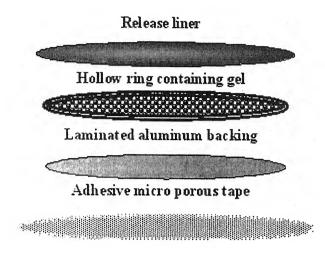


Figure 1. Fabrication design of reservoir type transdermal drug delivery system for piroxicam niosomal gel formulation.

Evaluation

Encapsulation efficiency

Weighed quantity of proniosomal gel (S2, S4, S6 and S8) were hydrated with saline and placed in a glass tube to which a Hi Media dialysis membrane was securely attached and dialyzed into 600 ml of saline (0.9%w/v) for 8h with three washings. The niosomes were collected and lysed using ethanol and suspended in 30% PEG 400 – phosphate buffer saline pH 7.4. The resulting solution was filtered and analyzed spectrophotometrically at 356 nm. Encapsulation efficiency (%EE) was calculated by the following equation:

 $\%EE = [(C_1-C_1)/C_1] \times 100\%$

Where C_1 is the total concentration of drug and C_1 is the concentration of free drug.

Scanning electron microscopy (SEM)

Proniosome powders were affixed to double-sided carbon tape, positioned on an aluminum stub and excess powder removed. The stubs were stored under vacuum overnight. The samples were sputter-coated with gold. Electron micrographs were obtained using scanning electron microscope operating at 15 kV accelerating voltage (LEO 435 VP 501B Electron Microscopy Ltd, UK).

In vitro skin permeation studies

The full-thickness abino Wistar rat skin was used for the permeation experiments. After removing the hair with a clipper, the skin was rinsed with physiological saline and clamped between the donor and the receptor chamber of Keshary Chien diffusion cell with the stratum corneum surface facing the donor compartment of vertical diffusion cell. The effective diffusion area of the cell was 2.0 cm² and had a receptor volume of 22 ml. The receptor chamber was filled with 30%v/v PEG 400 in phosphate buffer saline pH 7.4. The diffusion cell was maintained at 37 ± 1°C and the solution in the

receptor chambers was stirred continuously at 600 rpm with the help of magnetic bead. 2 g of niosomal gel of piroxicam was gently placed in the donor chamber and spread evenly. 2 ml of the solution in the acceptor chamber was removed for drug content determination and replaced immediately with an equal volume of receptor media. Drug concentration was determined UV spectrophotometrically at 356 nm (y = 0.0316x + 0.0066, R^2 = 0.9997).

In vivo anti-inflammatory studies

Experiments were approved by the Animal Ethics Committee of the University. Male Wistar rats $(220-250~\mathrm{g})$ were assigned to weight-balanced groups (n=6). The experimental groups received the different formulations, while the control group was treated with placebo only. In the experiment, 2 g of the different formulations (S6L, S6LM and S6LS) were applied over 9 cm² as transdermal patch on the dorsal skin after removing the hair with a clipper. After 2 h, 0.05 ml of a 0.5% carrageenan suspension was injected into the subplantar area of the left hind paw. The activity was measured by measuring the changes in paw volumes with a screw gauge (Mitutoyo, Kanagawa, Japan) 4 h after carrageenan injection. The right hind paw served as control was treated with physiological saline solution without carrageenan (Alol, 1993). The degree of paw swelling and inhibition in inflammation was calculated as:

Swelling (%)=
$$\frac{V_t - V}{V} \times 100$$

Where, V_t is the volume of the carrageenan-treated paw, V is that of the non-treated paw.

Inibition(%)=
$$\frac{S_c - S_t}{S_c} X 100$$

Where, S_c is the swelling of the control paw, S_t is that of the test formulation treated paw.

Statistical analysis

The results were analyzed by Student's t-test using Statistica for Windows (Version 5.0) from StatSoft, Inc., USA. The results were evaluated at probability level of p < 0.05.

RESULTS AND DISCUSSION

Among the niosomes prepared with spans, S4 and S6 showed maximum percentage entrapment that is, 90.4 and 94.8% respectively. The particle size analysis revealed that niosomes from S4 were larger as compared to those of S6 (Figure 2). Vesicles with smaller diameter are believed to better permeate through the skin as smaller vesicles tend to fuse readily. Niosomes of S6 were smaller in size, demonstrated higher entrapment efficiency and higher surface area as compared to that of S4. Niosomes of S2 demonstrated low entrapment efficiency and was not taken up for further study. It was observed that niosomes of Span 40 produced niosomes of larger size but in case of piroxicam vesicles from Span 60 had higher entrapment efficiency (Figure 3) and were

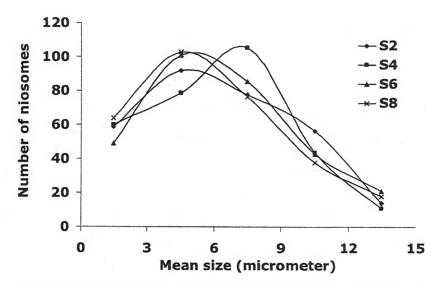


Figure 2. Mean size distribution of piroxicam niosomes prepared from S2, S4, S6 and S8.

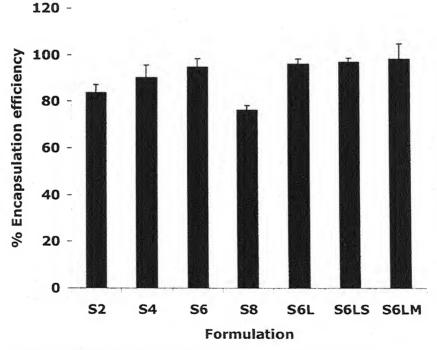


Figure 3. Encapsulation efficiency of various piroxicam niosomes (%EE±S.D.).

therefore selected, this probably could be credited to its high transition temperature and low permeability. Attempt was also made to incorporate lecithins. Egg lecithin was added and it was found to enhance drug entrapment. Incorporation of lecithin is also justified as it acts as

permeation enhancers. Incorporation of lecithin further enhanced the percent drug entrapment to 96.1%. Incorporation of lecithin leads to vesicles of smaller size due to increase in hydrophobicity which results in reduction of vesicle size (Alsara et al., 2005). There is probably for-

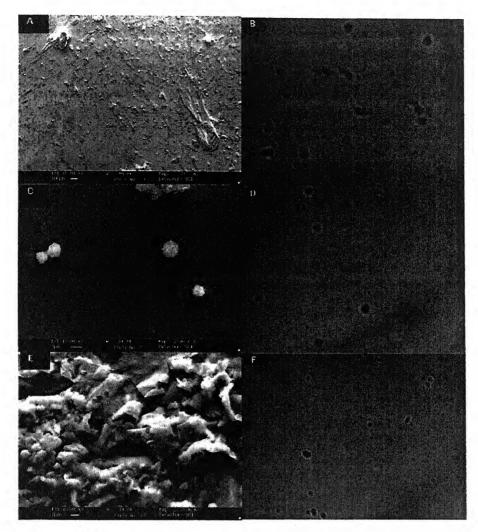


Figure 4. SEM images of proniosomes and polarized microscopic view of niosomes (40x): conventional (A & B), maltodextrin based (C & D), sorbitol based (E & F).

mation of more compact and well organized bilayers which prevents the leakage of drug (Fang et al., 2001).

In the present study attempt was also made to formulate niosomes from proniosomes formed on maltodextrin and sorbitol. Maltodextrin and sorbitol provided a base for preparing proniosomes. Percentage entrapment (Figure 3) observed was 97.2 and 98.6% respectively. Proniosomes prepared by conventional method were subjected to scanning electron microscopy (SEM) (Figure 4A). It was observed that preparing proniosomes on dry powder base was easier, provided the powder is not over wetted during the process. Preparing proniosomes on maltodextrin was comparatively easy as compared to sorbitol but it was necessary that the solution be incorporated in very small amounts and complete drying be ensured before further additions are made.

Maltodextrin is a polysaccharide; it has minimal solubility in organic solvents. Thus, it is possible to coat maltodextrin particles by simply adding surfactant in organic solvent to dry maltodextrin and evaporating the solvent. The maltodextrin particle morphology is preserved (Figure 4C), circular maltodextrin particles can be used for a significant gain in surface area. The higher surface area results in a thinner surfactant coating, which makes the rehydration process more efficient. The use of maltodextrin as the carrier in the proniosome preparation permitted flexibility in the ratio of surfactant and other components which can be incorporated.

Coating sorbitol results in a solid cake like mass (Figure 4E). It was necessary that the sorbitol bed be completely dry before further additions are made and making proniosomes with a reduced amount of sorbitol

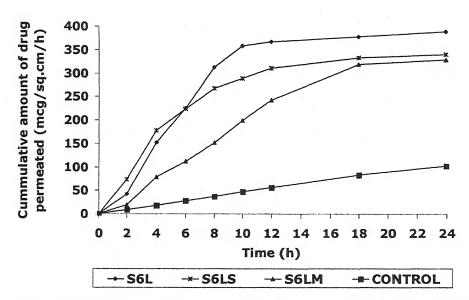


Figure 5. Cumulative amount of drug permeated through rat abdominal skin from different piroxicam niosomes.

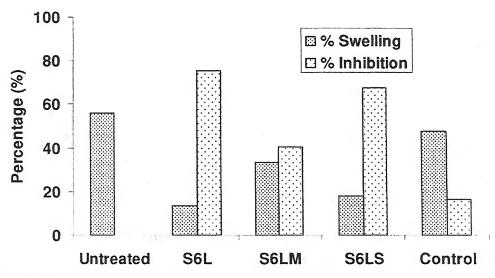


Figure 6. Anti-inflammatory studies of niosomal gel based piroxicam transdermal system.

was not only tedious but lead to niosomes with larger vesicle size. Addition of water leads to swelling of bilayers as well as vesicles due to interaction of water with polar groups of surfactant. In presence of excess of water there was complete hydration leading to formation of niosomes. Niosomes formed from conventional proniosomes, maltodextrin based and sorbitol based proniosomes are shown in (Figure 4B, 4D and 4F) respectively. The niosomes were visualized by polaroid

microscope (Nikon HFX-DX, Labophot Microscope, Germany).

The amount of drug permeated across rat abdominal skin was determined for piroxicam carbopol gel and niosomes formulated using span 60 and lecithin (S6L) and on maltodextrin (S6LM) and sorbitol as base (S6LS) is shown in Figure 5. The flux attained at the end of 24 h was 4.82, 35.61, 20.14 and 19.05 µg/cm²/h respectively and their enhancement ratios were 1.00, 7.39, 4.18 and

3.95 respectively. The incorporation of maltodextrin and sorbitol retarded drug release. There was significant (p<0.05) increase in flux over control preparation.

The adsorption and fusion of niosomes onto the surface of skin and the role played by the constituents of niosomes might facilitate drug permeation across skin. The interaction of niosomes with skin probably alters the barrier properties of stratum corneum thus enhancing permeation.

Lecithins and surfactants present in proniosomes have been reported to alter the structure of the stratum corneum. The intercellular lipid barrier in the stratum corneum gets fluidized and becomes permeable (Barry, 2001; Ogiso et al., 1996) thus increasing the permeation of drugs. Fusion of noisome vesicles to the surface of skin, results in higher flux of the drug due to direct transfer of drug from vesicles to the skin (Barry, 2001).

The concentration of cholesterol and lecithin are important as they tend to affect the morphology of the vesicles. An alteration in their composition leads to disruption of vesicles which leads to leakage of free drug before fusion of the vesicles with the skin.

The results of the anti-inflammatory studies (Figure 6) revealed that span 60 based lecithin vesicles (S6L) showed significant (p<0.05) reduction in paw swelling. The percent inhibition was found to be more than that of piroxicam carbopol gel. It is probable that there is enhanced drug delivery from lipid vesicles. The short fall seen with maltodextrin and sorbitol based formulations account for the slow release observed in in-vitro studies.

Conclusions

The in vitro permeation of piroxicam from proniosomes of various compositions and types of nonionic surfactants have been studied and evaluated. Piroxicam was successfully entrapped within the lipid bilayers of the vesicles with high efficiency. The experimental results suggest that either the vesicles fuse with the intercellular lipid of the stratum corneum and transfer the drug from vesicles to the skin and/or there might be penetration enhancement due to surfactants. Presence of lecithin probably aids the process. Proniosomes thus are capable of delivering piroxicam and probably other drugs also.

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Microemulsion-based hydrogel formulation for transdermal delivery of dexamethasone

Amrish Chandra, Pramod Kumar Sharma¹, Raghuveer Irchhiaya

Institute of Pharmacy, Bundelkhand University, Kanpur Road, Jhansi, Uttar Pradesh, India, ¹R. V. Northland Institute, Dadri, Gautam Budh Nagar, Uttar Pradesh, India

The purpose of this study was to construct a microemulsion-based hydrogel formulation for the transdermal delivery of dexamethasone. Almond oil, olive oil, linseed oil, and nutmeg oil were screened as the oil phase. A microemulsion-based system was chosen due to its good solubilizing capacity and skin permeation capabilities. The pseudoternary phase diagrams for microemulsion regions were constructed using various oils, egg lecithin as the surfactant, isopropyl alcohol (IPA) as the cosurfactant, and distilled water as the aqueous phase. Microemulsion gel formulations were prepared using Carbopol and filled into a reservoir-type transdermal system. The ability of various microemulsion formulations to deliver dexamethasone through the rat skin was evaluated *in vitro* using Keshary Chien diffusion cells. In order to enhance permeation, the skin was treated with an abrading gel (apricot seed powder in hydrogel base). The *in vitro* permeation data showed that microemulsions increased the permeation rate of dexamethasone compared with the control. The optimum formulation consisting of 0.1% dexamethasone, 10% olive oil, 70% egg lecithin:IPA (2:1), and water showed a permeation rate of $54.9 \,\mu\text{g/cm}^2/\text{h}$. The studied microemulsion-based hydrogel was stable toward centrifugation test and was nonirritating to the skin. The pharmacodynamic studies indicated that microemulsion based on nutmeg oil demonstrated a significantly (P < 0.05) higher anti-inflammatory potential. The nutmeg oil-based transdermal microemulsion gel system demonstrated 73.6% inhibition in rat paw edema. Thus, microemulsion-based transdermal systems are a promising formulation for dermal delivery of dexamethasone.

Key words: Carbopol, dexamethasone, hydrogel, microemulsion, transdermal

INTRODUCTION

Microemulsion is defined as a dispersion consisting of oil, surfactant, cosurfactant, and aqueous phase, which is a single optically isotropic and thermodynamically stable liquid solution with a droplet diameter usually within the range of 10-100 nm.[1] Microemulsions have several advantages, such as enhanced drug solubility, good thermodynamic stability, and enhancing effect on transdermal ability over conventional formulations.[2] There are several permeation enhancement mechanisms of microemulsions, such as an increased concentration gradient and thermodynamic activity toward skin and the permeation enhancement activity of the components of microemulsions.^[3] So far, much attention has been focused on the dermal delivery of drugs such as estradiol and lidocaine using microemulsions.[3,4] Recently, different hydrogel matrices such as carbomer 934, Carbopol, and carrageenan have been used to increase the viscosity of microemulsion for dermal application. [3.5-7] The addition of hydrogel matrix into the microemulsion resulted in the formation of the microemulsion-based hydrogel, which is more suitable for dermal application when compared with microemulsion.

Dexamethasone is a synthetic glucocorticoid^[9] widely used in inflammatory condition[10] and to enhance the normal immune response.[11] It is used as a therapeutic agent in alcohol withdrawal syndrome, [12] cerebral edema,[13] congenital adrenal hyperplasia,[9] nausea, and vomiting, especially associated with a high dose of anticancer agents,[14] high altitude disorder,[13] cerebral malaria, opportunistic mycobacterial infections, respiratory disorders, skin disorders, 191 and rheumatism.[15] Dexamethasone possesses most of the desirable physicochemical and biological properties, e.g. half-life of 2 and 5 h, plasma protein binding is about 67%, and a small daily dose ranging from 0.5 to 9 mg, while the associated drawbacks like hepatic first-pass effect of the drug and gastric irritation upon oral administration can also be overcome by transdermal delivery.[16] Researchers have also reported methodologies for increasing the transdermal

Address for correspondence:
Dr. Amrish Chandra, Institute of Pharmacy, Bundelkhand University,
Kanpur Road, Jhansi - 284 128, Uttar Pradesh, India.
E-mail: amrish_chandra@yahoo.com

The goal of this work was to develop a reservoir-type transdermal delivery system with the drug core being formed of a microemulsion-based hydrogel and to evaluate the permeation-enhancing potential of an abrading agent. The present study was focused on the screening of dexamethasone-loaded microemulsions and formulation of a microemulsion-based hydrogel.

MATERIALS AND METHODS

Materials

Dexamethasone was provided as a gift sample by Arbro Pharmaceuticals, New Delhi, India. Almond oil was procured from Fluka Chemicals Corp., Switzerland, and linseed oil and nutmeg oil were from Aldrich Chemicals Company, USA. Egg lecithin was purchased from HiMedia Laboratories, Mumbai, India, IPA from Ranbaxy Chemicals Pvt. Ltd., New Delhi, India, and olive oil and Carbopol 934 from CDH Pvt. Ltd., New Delhi, India. Hydroxy propyl methyl cellulose (HPMC K₁₅M) was gifted by Gattefosse, France, through Colorcon Asia Pvt. Ltd., Goa, India. All chemicals used were of analytical grade. Animal experiments were approved by the institutional animal ethical committee.

Pseudoternary phase diagram study

Almond oil, olive oil, linseed oil, and nutmeg oil were selected as the oil phase. Egg lecithin and IPA were selected as surfactant and cosurfactant, respectively. Distilled water was used as an aqueous phase. Surfactant-cosurfactant mixture (S:CoS) of different weight ratios (1:3, 1:2, 1:1, 1:0, 2:1, 3:1, and 4:1) were chosen. These S:CoS ratios were chosen in both increasing concentration of cosurfactant with respect to surfactant and increasing concentration of surfactant with respect to cosurfactant. Pseudoternary phase diagrams of oil, S:CoS, and aqueous phase were developed using the aqueous titration method under magnetic stirring at 20 rpm. Slow titration with the aqueous phase was performed to each weight ratio of oil and S:CoS. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions by virtue of their clarity and transparency. The physical state of the microemulsion was marked on a pseudo three-component phase diagram with the aqueous phase, oil phase, and mixture of surfactant and cosurfactant representing the three axes.

Screening of oils for microemulsions

To find out the suitability of oil to be used as the oil phase in microemulsion, the solubility of dexamethasone was determined. Microemulsions were prepared with each oil, S:CoS (almond oil–1:1, olive oil–1:3, linseed oil–3:1, and nutmeg oil–2:1), and water in a 1:7:2 ratio. Egg lecithin and

IPA were selected as surfactant and cosurfactant, respectively. Excess amount of dexamethasone was added to 2 ml of the selected microemulsions in 5 ml capacity vials. It was placed in a shaker water bath maintained at $37 \pm 1.0^{\circ}\text{C}$ for 72 h. The equilibrated samples were removed from the shaker, centrifuged, and the supernatant was filtered through Whatman filter no. 4. The concentration of dexamethasone was determined UV spectrophotometrically at 244 nm.

Preparation of the transdermal microemulsion-based hydrogel formulation of dexamethasone

Preparation of the microemulsion of dexamethasone Dexamethasone was added to the mixtures of oil and S:CoS with a varying component ratio as described in Table 1, and then the appropriate amount of water was added to the mixture drop by drop and the microemulsion containing dexamethasone was obtained by stirring the mixtures. All microemulsions were stored at $30 \pm 2^{\circ}$ C. Dexamethasone at 0.1% w/w was incorporated in all formulations.

Preparation of the microemulsion-based hydrogel of dexamethasone

Carbopol was selected as the gel matrix to prepare the microemulsion-based hydrogel formulation. Carbopol was slowly mixed with the microemulsion under stirring. After Carbopol had swelled, it was kept overnight to obtain the microemulsion-based hydrogel. The control formulation was prepared by adding 0.1% w/w dexamethasone to phosphate-buffered saline (PBS) at pH 7.4 and was gelled by the addition of Carbopol (1% w/w) and triethanolamine.

Fabrication of the reservoir-type patch of microemulsion gel of dexamethasone

Transdermal patches (reservoir type) of dexamethasone were fabricated by encapsulating the dexamethasone gel preparation within a shallow compartment made of a hollow ring-shaped device and drug-impermeable backing membrane (laminated aluminum foil). A microporous tape of a larger area was stuck onto the impermeable-backing membrane to bring the transdermal patch in intimate contact with the skin. The device was closed by a release liner on the open side. The various formulations were designated as C, AO, OO, LO, and NO for formulations prepared from distilled water (control), almond oil, olive oil, linseed oil, and nutmeg oil, respectively.

Preparation and method of application of the abrasive gel The abrasive gel was prepared by heating distilled water to 80°C and adding to it crushed apricot seeds (Prunus armeniaca). The polymer (HPMC) 2% w/w was then added slowly and allowed to disperse uniformly. It was then cooled to form a viscous gel into which was entrapped crushed apricot seed pieces.

One gram of the gel was topically applied with the help of the index finger on the depilated site of application of formulation and was gently massaged onto the skin for a period of 2 min. The crushed apricot pieces were dusted off with a soft cloth.

The above-mentioned formulations were also applied after pretreatment of the site of application of the patch by an abrading gel and these were designated as CA, AOA, OOA, LOA, and NOA for formulations prepared from distilled water (control), almond oil, olive oil, linseed oil, and nutmeg oil, respectively.

Characterization of the dexamethasone-loaded microemulsion preparation

Droplet size determination

The droplet size distribution and the average droplet size of the microemulsion were measured using a Zetasizer Nano ZS (Malvern Instruments, UK) using water as the dispersant at 25°C.

Viscosity measurements

AO3

EQ1

AQ4

The viscosity measurements were performed at $25 \pm 0.1^{\circ}$ C using a Brookfield viscometer DV-II+ Pro (Middleboro, MA, USA) (Spindle: T-bar and torque >30%).

Stability of the microemulsion-based hydrogel

The stability of the studied microemulsion-based hydrogel containing dexamethasone was studied via clarity and phase separation observations. The centrifuge tests were carried out to assess the physical stability by subjecting the microemulsion to centrifugation at 3000 rpm for 15 min (RCF≈ 1200).

Determination of dexamethasone concentration in the receptor media

The drug content in the diffusion media, i.e. PEG 400 and PBS pH 7.4 (20:80), was measured UV spectrophotometrically at 244 nm. The assay was linear in the concentration range of 1–30 μ g/ml for dexamethasone (y = 0.0395x + 0.0023, R^2 = 0.9999).

In vitro skin permeation studies

The full-thickness rat skin was used for the permeation experiments. After the hair was removed with a depilatory, the skin was rinsed with physiological saline and then washed with PBS (pH 7.4). The skin was clamped between the donor and the receptor chamber of a Keshary Chien diffusion cell with an effective diffusion area of $1.0\,\mathrm{cm^2}$ and 11-ml receptor cell volume. The receptor chamber was filled with freshly prepared 20% PEG 400 in PBS (pH 7.4). The diffusion cell was maintained at $37\pm2\%$ C and the solution in the receptor chambers was stirred continuously with a magnetic bead. Two grams of the microemulsion-based hydrogel was placed in the donor chamber. In case of pretreatment with abrading gel, the skin was clamped and to it was applied 1 g of the abrading gel and was massaged onto the skin for 2 min. The crushed apricot seed debris was dusted off with a soft cloth, the donor chamber

was mounted, and the experiment was carried as outlined. At 1, 2, 4, 6, 8, 10, 12, 20, 22, and 24 h, 2 ml of the sample from the acceptor chamber was removed for determination of the drug concentration and replaced immediately with an equal volume of the receptor fluid. All experiments were performed in quadruplicate. The cumulative amount of dexamethasone permeated through the rat skin was plotted as a function of time. The permeation rate (flux) of dexamethasone at the steady state (J_{ss} , $\mu g/cm^2/h$) and the lag time (T_{lag} , h) were calculated from the slope and the intercept of the straight line obtained by plotting the amount of dexamethasone permeated versus time in steady state conditions, respectively. Permeability coefficient (Kp. cm/h) was calculated by dividing the flux obtained by the initial concentration of drug in the donor compartment. The enhancement ratio (ER) was calculated from the following equation:

$$ER = \frac{Jss of test formulation}{Jss of control formulation}$$

Pharmacological studies

Skin irritation study

Various preparations, when applied dermally, might elicit skin irritation. Therefore, to assess the skin-sensitizing potential, dexamethasone patch was applied onto the dorsal skin of albino Wistar rats. The animals were housed in polypropylene cages, with free access to standard laboratory diet and water. Animals were acclimatized for at least 7 days before experimentation. The dorsal abdominal skin of the rats was shaved 24 h before study. The formulations were applied and the site of application was occluded with gauze and covered with a nonsensitizing microporous tape. Erythema values for formulations with and without pretreatment with abrading gel were recorded. The patch was removed after 24 h and the score of erythema was recorded as follows: mild erythema—1; moderate erythema—2; severe erythema—3.[27]

In vivo anti-inflammatory studies

Wistar albino rats (220–250g) were assigned to weight-balanced groups (n=4). The experimental groups received the different formulations while the control group was treated with placebo only. Two grams of the different formulations were spread over $9\,\mathrm{cm}^2$ of dorsal skin after removing the hair with a clipper. After $2\,\mathrm{h}$, $0.05\,\mathrm{ml}$ of a 0.5% carrageenan suspension was injected into the subplantar area of the left hind paw. The activity was measured by measuring the changes in paw volumes with a screw gauge (Mitutoyo, Kanagawa, Japan) $4\,\mathrm{h}$ after carrageenan injection. The right hind paw served as a control and was treated with physiological saline solution without carrageenan. [29] Four hours after the carrageenan injection, the degree of paw swelling was calculated as follows:

Swelling (%) =
$$\frac{V_t - V}{V} \times 100$$

where, V₁ is the volume of the carrageenan-treated paw and V is that of the nontreated paw.

$$lnibition(\%) = \frac{S_c - S_t}{S_c} \times 100$$

where, S_c is the swelling of the control paw and S_t is that of the test formulation-treated paw.

Statistical analysis

All skin permeation experiments were repeated four times and data were expressed as the mean value \pm SD. The results were analyzed by Student's *t*-test using Statistica for windows (version 5.0) from StatSoft Inc., USA.

RESULTS AND DISCUSSION

Phase studies

AQ5

The aim of construction of pseudoternary phase diagrams was to find out the existence range of microemulsions. The phase diagrams of the four selected systems are presented in Figure 1. It can be seen that the oil/water (o/w) microemulsion region was slightly larger for olive oil and nutmeg oil while linseed oil and almond oil had comparatively smaller areas. However, all ternary phase diagrams revealed that at least a 10% w/w oil phase could be incorporated using egg lecithin

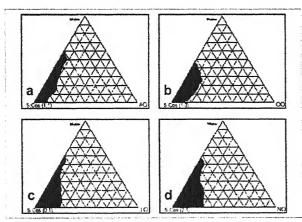


Figure 1: The pseudoternary phase diagrams of the oil, surfactant cosurfactant mixture, and water system at 1:7:2 weight ratios of oil, egg lecithin—isopropyl alcohol mixture and distilled water at 25°C. (a) Almond oil, (b) olive oil, (c) linseed oil, and (d) nutmeg oil (shaded portion represents the microemulsion region)

and IPA as surfactant and cosurfactant, respectively. The dark-shaded region in the phase diagram marks the clear transparent microemulsion phase.

There was occurrence of distinct phases, the immiscible phase, the translucent phase, and the clear transparent phase. The conversion from turbid phase to oil-in-water (o/w) microemulsion phase was observed. The major region on the phase diagram represents the turbid and the conventional emulsion. The microemulsion region changed slightly in size with the increasing ratio of surfactant to cosurfactant. It was observed that the amount of oil that could be incorporated varied with the type of oil and also on the ratio of S:CoS. The S:CoS ratio yielding largest areas was selected for each oil. The selected phase diagrams are shown in Figure 1.

Because the order of the addition of the ingredients has a significant influence on the preparation of microemulsions, [30,31] the dexamethasone was first dissolved/dispersed in the oil phase and to it was added the S:CoS mixture of selected ratio at defined concentrations and then slowly titrated with water. The concentrations of the components are represented in Table 1.

Solubility of dexamethasone

In order to develop microemulsion formulations for dermal delivery of poorly water-soluble dexamethasone, the optimum oil was selected by determining the concentration of dexamethasone that would dissolve. Four different microemulsions were prepared using oil, S:CoS (egg lecithin–IPA) mixture, and distilled water in a 1:7:2 ratio. The solubility of dexamethasone in microemulsions of various oils is reported in Table 2.

The solubility of dexamethasone was highest in microemulsions of olive oil (14.2 \pm 0.36 mg/ml), followed by nutmeg oil, almond oil, and linseed oil. The solubility of dexamethasone in microemulsions of various oils decreased slightly when compared with that in olive oil. The addition of egg lecithin and IPA to oil would probably positively influence drug solubility. It was therefore inappropriate to study the solubility of dexamethasone in the oil phase. Microemulsions were therefore choosen for assessing the solubility. Dexamethasone demonstrated high solubility in microemulsions of various oils. As per the observed solubility, olive oil and nutmeg oil could be the most appropriate

Table 1: Compositions of the selected microemulsion formulations (% w/w)

Microemulsion components (% w/w)	Formulation codes				
	С	AO	00	LO	NO
Dexamethasone	0.1	0.1	0.1	0.1	0.1
Oil	???	10	10	10	10
Egg lecithin	???	35	17.5	52.5	46.6
IPA	???	35	52.5	17.5	23.3
Distilled water q.s.	100	100	100	100	100
Vehicle	Distilled water	Almond oil	Olive oil	Linseed oil	Nutmeg oil

oils for the development of the microemulsion. In order to verify the selection of the oil, the *in vitro* skin permeation rate of dexamethasone from the microemulsions containing dexamethasone, oil, egg lecithin—IPA mixture, and water was also determined.

Preparation of the transdermal microemulsion-based hydrogel formulation of dexamethasone

After addition of carbomer 934 followed by addition of triethanolamine, it was observed that the microemulsion structure was disturbed. However, a clear gel could be obtained by omitting triethanolamine. The microemulsion-based hydrogels with 1.0% w/w Carbopol were stable at 40°C. No phase separation and degradation was observed during 3 months. The microemulsion-based hydrogel with 1.0% Carbopol formulated as a reservoir-type transdermal device could be applied to the skin as such or after treating the area of the skin with an abrading gel.

Characterization of the dexamethasone-loaded microemulsions

The droplet size of all microemulsions ranged from 4 to 13 nm. The droplet size of the microemulsion was found to vary with the oil. Olive oil and nutmeg oil produced microemulsions of 10–13 nm in diameter while almond oil and linseed oil microemulsions had a droplet size of 4–6 nm. The small droplet sizes are very much a prerequisite for drug delivery as the oil droplets tend to fuse with the skin thus providing a channel for drug delivery.

The viscosities of the microemulsion-based Carbopol gels ranged from 160 to 200 Poise for the ME gel and the conductivities were in the range of 8×10^{-6} – 10×10^{-6} S/cm. The conductivities of the microemulsion revealed that water was the external phase and the microemulsion was o/w type. The microemulsion formulations had pH values varying from 4.1 to 4.7 [Table 3].

Incorporation of different oils did not greatly affect the pH of the microemulsions. The centrifuge test showed that the microemulsion had good physical stability.

Table 2: Solubility of dexamethasone in microemulsions of various oils at 25°C (mean \pm SD, n = 4)

Microemulsion oil (S:CoS)	Solubility (mg/ml)
Almond oil (1:1)	10.6 ± 0.1
Olive oil (1:3)	14.2 ± 0.3
Linseed oil (3:1)	9.4 ± 0.2
Nutmeg oil (2:1)	11.8 ± 0.4

In vitro skin permeation studies

The high permeation rate of microemulsions might be attributed to several factors. Microemulsions could act as a drug reservoir. where drug is released from the inner phase to the outer phase and then further on to the skin. [3] Secondly, because of the small droplet size, droplets settled down to close contact with the skin and a large amount of the oil phase in the microemulsions might penetrate into the skin. [3] In addition, because of the small droplet diameters of the microemulsions, the probable mechanism may also be the permeation of dexamethasone directly from the droplets into the stratum corneum without microemulsion fusion to the stratum corneum and subsequent permeation enhancement. The concentration of the mixtures of egg lecithin and IPA was 70% w/w in all preparations but their ratio probably influenced the permeation rates. Higher fluxes were observed for OO and NO formulations (40.740µg/cm²/h and 36.0 μ g/cm²/h). These microemulsion had S:CoS ratio of 1:3 and 2:1, respectively. Despite the fact that nutmeg oil had a larger droplet size, its permeation rate was not much affected, a phenomenon which needs to be analyzed. A microemulsion gel-based system of almond oil and linseed oil had a lower flux, i.e. 28.9 and 21.8 μ g/cm²/h, respectively. IPA has a strong permeation enhancing effect by virtue of its ability to enhance the solubility of dexamethasone in the skin by disrupting the lipid bilayer of the skin.

The lecithin and IPA present act not only as a surfactant and stabilize the microemulsion but they also act as permeation enhancers. They probably interact with the intercellular lipids in the stratum corneum. IPA tends to fluidize the lipids thus increasing the permeation of dexamethasone.

Figure 2 shows the release profile of microemulsion formulations of dexamethasone in different oils and control preparations. Two sets of experiments were performed, i.e. without pretreatment of the skin with an abrading hydrogel (C, AO, OO, LO, and NO) and after pretreatment with an abrading hydrogel (CA, AOA, OOA, LOA, and NOA). It was observed that use of an abrading gel significantly increased the permeation rate of dexamethasone across the rat skin (*P* < 0.05).

The use of an abrading hydrogel of crushed apricot seed probably assisted in enhancing the drug permeation by removing the upper dead layer of skin, i.e. the stratum corneum. There was a 1.3–1.4-times increase in the permeation of dexamethasone across the rat skin after abrading the skin compared with the same formulation when applied without pretreatment. The flux of dexamethasone

Table 3: Physicochemical parameters of tested dexamethasone-loaded microemulsions

Microemulsion oil	Droplet size (nm)	Viscosity (Poise)	Conductivity (S/cm, 10 ⁻⁶)	nH
Almond oil	4.2	172.5	9 1	4.5
Olive oil	10.8	162.9	8.9	4.1
Linseed oil	5.0	193.8	8.2	4.4
Nutmeg oil	12.7	166.3	9.6	4.7

TQ1

Q6

formulation NOA increased from 36.0 to $50.2\,\mu\text{g/cm}^2\text{/h}$, while that of formulation OOA increased from 40.7 to 54.9 $\mu\text{g/cm}^2\text{/h}$. Formulation OO showed an ER of 2.2 without pretreatment as compared with 1.2, 1.6, and 1.9 for LO, AO, and NO. The ERs and permeability coefficient values are reported in Table 4.

TQ1

TQ1

TQ1

TQ1

A maximum flux of $54.9\mu g/cm/h$ was achieved for formulation OOA after pretreatment with abrading gel and the total amount of drug delivered across the rat skin was $1283.5\mu g/cm$. The observed lag time reduced from 0.8 to 0.6h after pretreatment of skin. There was a significant (P < 0.05) reduction of lag time on pretreatment of the skin with the abrading hydrogel.

This may be probably because the primary pathway of transdermally delivered drugs is paracellular, i.e. around the cells than through the elastin. Elastin is composed of collagen and hyaluronic acid and other lipids, which occupies the interstices between the cells of the top-most layer of the skin (i.e. the epidermis, including, e.g. stratum corneum, lucidum, granulosum, spinosus) and must be dissolved and/ or disrupted in order for the drug to be able to transverse

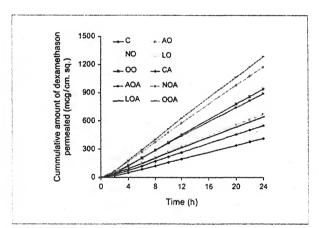


Figure 2: Permeation profiles of dexamethasone through the excised rat skins from the microemulsion-based gel formulations with different oils (mean \pm SD, n = 4)

through the viable skin to the subcutaneous tissues where the cutaneous plexi of the capillary net can be reached. Permeation of oil into the lipid layers of the skin may lead to the change in the lipid barrier properties. Further disruption of the stratum corneum barrier by the abrading agent might enhance the permeation by removing the upper dead stratum corneum.

In case of human subjects, it is desirable that the formulation delivers 0.5–9 mg of dexamethasone in 24 h. Therefore, the desired plasma levels can be achieved by employing a patch of size 6.8 cm².

Pharmacological studies

Skin irritation studies

The skin irritation studies revealed that all formulations were nonsensitizing and safe for use.

In vivo anti-inflammatory effect

The microemulsion-based transdermal system showed a significant (P < 0.05) response in the carrageenan-induced paw edema inflammation model. The formulation demonstrated significantly improved activity compared with that of the control (P < 0.05) and improvement was measured in terms of paw edema volume 4 h after carrageenan injection.

The anti-inflammatory potential revealed that that there was a significant reduction of paw swelling when compared with the results from the nonabraded skin. There was 62.2 and 73.6% reduction of paw volume for the microemulsion gel-based transdermal device for OOA and NOA, respectively, when applied after the pretreatment of the site of application with the abrading gel. NOA demonstrated a significant reduction (P < 0.05) in paw edema compared with OOA.

Comparative reductions in edema volume with respect to control are shown in Figure 3. A 32.7% decrease in paw inflammation was recorded when the control formulation was applied dermally after abrading the skin with the abrading gel thus clearly indicating the role of the abrading gel in enhancing the permeation. The effect of nutmeg oil over that of olive oil was more pronounced. NOA microemulsion

Table 4: The permeation parameters of the dexamethasone-loaded microemulsion-based gel transdermal system

Microemulsion formulation code	Enhancement ratio		Permeability coefficient	Lag time
	w.r.t. control formulation v	r.t. unabraded application	Kp10 ⁻⁴ (cm h ⁻¹)	T _{iag} (h)
C	1.0	1.0	18.1	1.1
CA	1.3	1.3	23.7	0.7
AO	1.6	1.0	28.9	0.7
AOA	2.0	1.3	37.9	0.4
00	2.2	1.0	40.7	0.8
OOA	3.0	1.3	54.9	0.2
LO	1.2	1.0	21.8	0.8
LOA	1.5	1.2	27.4	0.5
NO	1.9	1.0	36.0	0.7
NOA	2.7	1.4	50.2	0.5

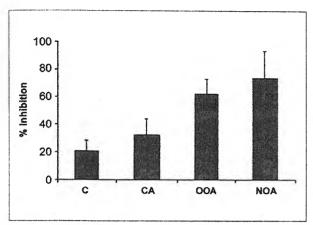


Figure 3: Anti-inflammatory activity of the microemulsion-based transdermal formulations of dexamethasone (mean \pm SD, n = 4)

formulation demonstrated 11.7% higher activity as compared with the olive oil-based microemulsion formulation. The transdermal microemulsion gel-based system thus has due potential to deliver dexamethasone at therapeutically effective concentrations.

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EQ2

Effect of alcohols and enhancers on permeation enhancement of ketorolac

Amrish Chandra, Pramod Kumar Sharma¹, Raghuveer Irchhiaya

Institute of Pharmacy, Bundelkhand University, Kanpur Road, Jhansi, Uttar Pradesh, India, R. V. Northland Institute, Dadri, Gautam Budh Nagar, Uttar Pradesh, India

reservoir-type transdermal patch for the delivery of ketorolac was studied. The low permeability of the skin is the rate-limiting step for delivery of most of the drugs. Studies were carried out to investigate the effect of pH, alcohols, and chemical permeation enhancers on the *in vitro* permeation of ketorolac. The reservoir core of the transdermal patch was filled with the hydrogel of a nonionic polymer, methocel $K_{15}M$ (hydroxyl propyl methylcellulose, HPMC) formulated at an optimized pH of 5.4. Enhanced *in vitro* permeation was achieved after the incorporation of the alcohols. Higher enhancement was produced by short-chain alcohols like ethanol and isopropyl alcohol (IPA). Propylene glycol (PG) along with other alcohols, viz. n-propanol, n-butanol, and n-pentanol, lagged behind. An exponential rise in permeation was observed in flux with an increase in the concentration of IPA. At 25% w/w IPA concentration, the observed ketorolac flux was 18.04 μ g/cm²/h. Terpene containing eucalyptus oil was studied to determine its permeation enhancement capability. The increase in the concentration of eucalyptus oil enhanced the drug permeation and a maximum flux of 66.38 and 90.56 μ g/cm²/h was achieved at 10 and 15% w/w concentrations. The anti-inflammatory potential of the transdermal formulation was evaluated on a carrageenan-induced paw edema model, with 41.67% inhibition at 6 h. The skin irritation potential was evaluated by the Drazie test and the formulations prepared were found to be safe. The reservoir-type transdermal patch for the delivery of ketorolac appeared to be feasible for delivering ketorolac across the skin.

Key words: Enhancer, eucalyptus oil, isopropyl alcohol, ketorolac, permeation, transdermal

INTRODUCTION

Ketorolac (administered as tromethamine salt), a prostaglandin synthetase inhibitor is a nonsteroidal anti-inflammatory drug with potent analgesic and moderate anti-inflammatory activities. [1] Ketorolac has been studied clinically and its efficacy and safety as an analgesic in postoperative pain and cancer have been established. Administered as oral and injectable formulations, it has shown a high analgesic potency almost equivalent to that of morphine. [2] Unlike narcotic analgesics, ketorolac does not alter the gastric motility or the hemodynamic variables or adversely affect the respiration, nor is it associated with abuse or addiction potential as in the case of narcotic analgesics; therefore, ketorolac is a relatively more favorable therapeutic agent for the management of moderate to severe pain. [3]

Ketorolac thus has substantial clinical potential and developing an alternative dosage form that is easy to administer, is painless, noninvasive, easy to comply, and avoids first-pass metabolism is worthwhile. The transdermal route encompasses all the above advantages.

Despite these advantages, only a limited number of drugs can be administered percutaneously due to low skin permeability of most drugs through the skin. The penetration through the stratum corneum is the rate-limiting step for the delivery of most of the drugs. To overcome this problem, vehicles, [4] penetration enhancers, [5] ultrasound, [6] and electrotransport [7]_facilitated transdermal systems have been attempted in the development of a transdermal delivery system of ketorolac. The prodrug approach has also been investigated for enhanced dermal delivery. [8,9] However, the most widely used technique involves use of chemical penetration enhancers or solvents that modify the thermodynamic activity.

In the present study, we investigated the effects of pH, alcohols, and chemical penetration enhancers on the *in vitro* permeation of ketorolac from hydrogel gel formulation across rat abdominal skin to examine the feasibility of developing a transdermal system.

Address for correspondence;

Dr. Amrish Chandra, Institute of Pharmacy, Bundelkhand University,
Kanpur Road, Jhansi - 284 128, Uttar Pradesh, India.

E-mail: amrish_chandra@yahoo.com

MATERIALS AND METHODS

Ketorolac (tromethamine salt) was obtained as a gift sample from Ranbaxy Laboratories, Devas, India. HPMC (Methocel® K, M) was gifted by Colorcon Asia Pvt. Ltd., Goa, India, n-propanol, isopropyl alcohol, n-butanol, n-pentanol, and PG and eucalyptus oil were purchased from Central Drug House. New Delhi, India. Ethanol was procured from E. Merck (India) Ltd., Mumbai, India. Other chemicals and reagents used were of analytical grade. The experimental protocol was approved by the institutional animal ethical committee.

Preparation of the ketorolac gel system and fabrication of the reservoir-type patch

The ketorolac gel system was prepared in phosphate-buffered saline (PBS) solution by dissolving ketorolac and adding methocel K, M (HPMC) with continuous stirring so as to uniformly disperse the polymer. Permeation enhancers were mixed with the vehicle before adding the polymer. The gel was kept overnight at an ambient temperature in a tightly closed container to allow uniform gelling (cold dispersion method).

Transdermal patches (reservoir type) of ketorolac were fabricated by filling the ketorolac gel preparation (0.25 g/cm²) within a shallow compartment made of a hollow ring-shaped device and drug impermeable backing membrane (laminated aluminum foil). A microporous adhesive tape of a larger area was stuck onto the impermeable backing membrane to bring the transdermal patch in intimate contact with the skin. The device was closed by a release liner on the open side |Figure 1|.

In vitro skin permeation studies of ketorolac through the excised rat dorsal skin

The dorsal abdominal skin of Sprague-Dawley rats was clamped between the donor and the receptor chamber, with the stratum corneum surface facing the donor compartment

Release liner Hollow ring containing gel Laminated aluminum backing

Figure 1: Design of the reservoir-type patch for transdermal administration of ketorolac

of the modified Keshary Chien diffusion cell after removing the hair with a clipper. The effective diffusion area of the cell was 2.0 cm² and had a receptor volume of 11 ml. The receptor chamber was filled with freshly prepared PBS solution of pH 7.4. The diffusion bath was maintained at 37 ± 1°C and the solution in the receptor chamber was stirred continuously with the help of a magnetic bead. Ketorolac gel (0.5g) was gently placed in the donor chamber and was spread evenly on the rat abdominal skin. The donor compartment was covered with Parafilm M® to prevent evaporative loss. One milliliter of the solution in the acceptor chamber was removed for drug content determination and replaced immediately with an equal volume of fresh PBS solution pH 7.4. The drug concentration was determined UV spectrophotometrically.

Analysis of in vitro drug release study samples

Samples withdrawn were filtered through Whatmann filter paper no. 42 and diluted whenever necessary. The samples were analyzed spectrophotometrically at λ_{max} 324 nm. The concentration of the drug was determined from the regression equation generated from the calibration curve, $y = 0.0285x + 0.0012, R^2 = 0.999.$

Effect of pH on in vitro skin permeation of ketorolac through the rat skin

The ketorolac gel system was prepared, consisting of 2% w/w ketorolac and 2% w/w methocel K₁₅M swelled in PBS solution of defined pH by the cold dispersion method. Three different PBS solutions of pH 5.4, 6.4, and 7.4 were employed for preparing the formulation.

Effect of permeation enhancers on the in vitro skin permeation of ketorolac through the rat skin

The ketorolac gel system was prepared, which consisted of 2% w/w ketorolac and 2% w/w methocel K,5M in PBS solution at an optimized pH of 5.4 by the cold dispersion method. Various alcohols, viz. ethanol, n-propanol, IPA, n-pentanol, n-butanol, and PG, were evaluated at a 10% w/w concentration. The selected alcohol was further optimized at 10, 15, 20, and 25% w/w concentration. The enhancement potential of eucalyptus oil (5, 7.5, 10, and 15% w/w) was further studied at the optimized alcohol concentration in order to determine the most preferred composition for the transdermal gel system.

Skin irritation studies

The transdermal patch of ketorolac gel was applied onto the dorsal skin of the Wistar rats (220-250 g), which was shaved 24 h before the study. The site of application was occluded with gauze and covered with a nonsensitizing microporous adhesive tape. After 24 h, the gel was removed and the score of erythema was determined by the Drazie test[10] as follows: 0 - no erythema; 1 - mild erythema; 2 - moderate erythema;

3 - severe eryhtema.

Anti-inflammatory studies

The anti-inflammatory efficacy was evaluated by carrageenaninduced paw inflammation in the Wistar rat (approx. 220-250 g). The rats were assigned to treatment groups so that each group was weight balanced. Ketorolac was administered via the oral (2 mg/kg) and transdermal routes 1h before carrageenan injection. The transdermal patch was securely adhered over the depilated dorsal abdominal skin (9 cm²). The rats received an intraplantar injection of 0.05 ml of 0.5% w/v carrageenan suspension into the left hind paw subcutaneously by inserting the needle into the central part of the paw. The paw volume was measured and compared with that found in animals treated with carrageenan alone. The right hind paw, which served as the control, was treated with physiological saline solution without carrageenan.[11] The inflammatory response was determined by measuring the changes in paw volumes with a screw gauge (Mitutoyo, Kanagawa, Japan) at 0, 2, 4, 6, 8, and 10 h after the carrageenan injection.

The area under the curve (AUC) was determined by the trapezoidal method.

Data analysis

The *in vitro* skin flux was determined by Fick's law of diffusion, considering the transport of drugs across the skin barrier as a process of passive diffusion. J_{ss} , the skin flux ($\mu g/cm^2/h$), was determined from the slope of the linear portion of the cumulative amount permeated per unit area versus the time plot. The lag time (T_{lag} h) was determined by extrapolating the linear portion of the curve to the abscissa. Kp, the permeability coefficient (cm h⁻¹), was determined from the equation^[12]:

$$Kp = J_{ss}/C_{o}$$

Co is the donor phase concentration.

The enhancement ratio (ER) was calculated from the following equation:

ER = Jss of test gel / Jss of control gel

Statistical analysis

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The results were analyzed by Student's t-test (paired, two tailed) using Statistica for Windows (Version 5.0) from StatSoft Inc., USA. The results were evaluated at the probability level of P < 0.05.

RESULTS AND DISCUSSION

Effect of pH on *in vitro* skin permeation of ketorolac through the rat skin

The effect of pH on gel containing 2% w/w of ketorolac and 2% w/w methocel $K_{15}M$ on the permeation rate of ketorolac was studied at pH 5.4, 6.4, and 7.4. From the results, it was concluded that higher permeation of ketorolac occurred at

lower pH [Figure 2]. Flux attained for the gel system prepared in pH 5.4, 6.4, and 7.4 was 1.24. 0.99, and 0.79 $\mu g/cm^2/h$ and the observed T_{lag} was 7.70, 8.99, and 9.60 h, respectively. The permeation of a molecule depends primarily on its solubility and its state (ionized/unionized). Because the drug was present at a subsaturation concentration and was completely soluble at all pH, it was probably the degree of the unionized to ionized fraction that affected the permeation rate across the skin. Thus, PBS solution of pH 5.4 was selected for further studies.

Effect of permeation enhancers on *in vitro* skin permeation of ketorolac through the rat skin

Various alcohols, viz. ethanol, n-propanol, IPA, n-pentanol, n-butanol, and PG, at 10% w/w were evaluated for their ability to enhance permeation of ketorolac. Ketorolac gels were prepared in a PBS solution of pH 5.4. ER was calculated by comparing the steady state flux of ketorolac from the alcohol-containing gel system and that of the gel system at an optimized pH. The release profile is presented in Figure 3. From the study, it was perceived that smaller chain alcohols were more efficient in enhancing the dermal permeation. The shift of the hydroxyl group away from the edge side to the center considerably increased the permeation. Highest permeation coefficients were observed for IPA and ethanol. The steady state flux achieved was 4.67 and 3.91 µg/cm²/h, respectively, but a reduced lag time of 3.26 h was noted for ethanol in comparison with 3.55h for IPA. Other alcohols, viz. n-propanol, n-butanol, and n-pentanol, showed a flux of 3.53, 3.18, and 2.71 µg/cm²/h, respectively. Despite the fact that ethanol demonstrated an enhanced diffusivity, higher Q24 (amount of drug permeated across the skin at the end of 24 h) was achieved for IPA. Alcohol as a solvent may extract some of the lipid fraction from within the stratum corneum, thereby enhancing permeation. Various findings also reveal that longer chain alcohols possess a higher enhancement potential toward lipophilic drugs[13] and poor enhancement toward hydrophilic drugs. [14] Reports concerning the efficacy of PG as a permeation enhancer are mixed. Evidence suggests at best only a very mild enhancement effect for molecules such as estradiol and 5-fluorouracil.[15] In case of ketorolac, a lag time of 5.68 h is observed with PG as the alcohol component of the gel system and the flux attained was 5.68 µg/cm²/h.

Increasing the concentration of IPA further enhanced the permeation of ketorolac [Figure 4]. An ER of 14.55 was attained at 25% w/w of IPA concentration. There was an exponential (1.9167 $e^{0.0895x}$, $R^2 = 0.9996$) rise in the ketorolac flux.

The effect of eucalyptus oil on the permeation of ketorolac from the transdermal gel system across the rat abdominal skin was investigated. Gels were prepared using ketorolac (2% w/w), methocel $K_{15}M$ (2% w/w), IPA (25% w/w), and eucalyptus oil at varying concentrations (5, 7.5, 10, and

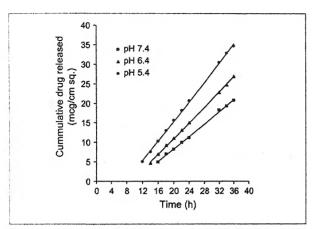


Figure 2: In vitro permeation profile of ketorolac through the rat abdominal skin from the gel system containing ketorolac (2% w/w) and methocel $K_{15}M$ (2% w/w) and formulated using phosphate-buffered saline solutions of different pH

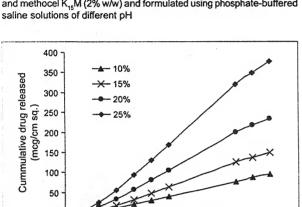


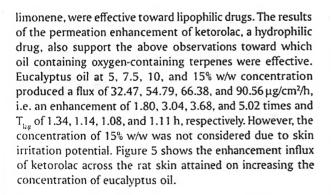
Figure 4: In vitro permeation profile of ketorolac across the rat abdominal skin from the gel system containing ketorolac (2% w/w), methocel $\rm K_{15}M$ (2% w/w), and isopropyl alcohol at varying concentrations formulated in phosphate-buffered saline solution of pH 5.4

10

Time (h)

15

20



These agents probably modify the solvent nature of the stratum corneum, improving drug partitioning into the tissue. Terpenes permeate the skin well.^[18] With loss of terpenes, which are generally good solvents, from a formulation, there could be an alteration of the thermodynamic activity of the

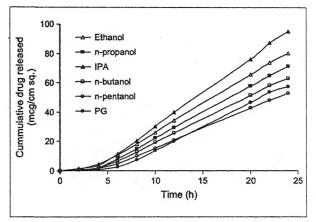


Figure 3: *In vitro* permeation profile of ketorolac through the rat abdominal skin from the gel system containing ketorolac (2% w/w) and methocel K₁₅M (2% w/w) and different alcohols at a 5% w/w concentration formulated using phosphate-buffered saline solution of pH 5.4

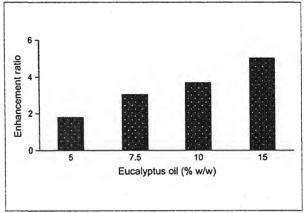


Figure 5: Enhancement ratio of ketorolac across the rat abdominal skin from the gel system containing ketorolac (2% w/w), methocel $K_{rs}M$ (2% w/w), isopropyl alcohol (25% w/w), and eucalyptus oil at varying concentrations formulated in phosphate-buffered saline solution of pH 5.4

15% w/w) in PBS solution of pH 5.4. The effectiveness of the enhancer was determined by comparing the steady state flux of ketorolac from the chemical enhancer containing gel system and that of the gel system at an optimized IPA concentration.

Essential oils like eucalyptus oil are reported to be effective penetration enhancers for 5-flouorouracil traversing in to the human skin *in vivo* with a maximum ER of 34-fold. ¹⁶¹ The principal terpene element within eucalyptus oil is 1, 8-cineole, a cyclic ether and proven enhancer, but its efficacy as an enhancer is mixed. Eucalyptus oil could not enhance the permeation of a lipophilic drug like estradiol. Similar results were also reported for the permeation of indomethacin, a lipophilic molecule. The study revealed that oxygen-containing terpenes (carvone, 1, 8-cineole) were ineffective where a hydrocarbon terpenes, especially

permeant. Terpenes may also modify drug diffusivity through the membrane and bring about a reduction of the lag time for permeation, indicating an increase in the diffusivity of the drug through the membrane following terpene treatment. X-ray diffraction studies have also indicated that 1, 8-cineole disrupts the stratum corneum bilayer lipids. ^[19] The ketorolac gel system consisting of ketorolac (2% w/w), methocel K₁₅M, (2% w/w), IPA (25% w/w), and eucalyptus oil (10% w/w) in PBS solution of pH 5.4 was selected as the optimized component composition of the gel system for transdermal delivery of ketorolac.

Skin irritation studies

The results of the skin irritation studies based on the visual observation score suggest that the formulations were safe to be applied on the skin. The scores for transdermal formulations containing eucalyptus was between 0 and 2. Eucalyptus oil at 15% w/w showed a slight increase in erythema.

Anti-inflammatory studies

The formulation showed a prominent increase in activity in the carrageenan-induced paw inflammation model. Figure 6 represents the anti-inflammatory activity after oral administration and after application of the transdermal formulation. Ketorolac transdermal patch formulation demonstrated a significant anti-inflammatory potential as compared with the control (P < 0.05). The anti-inflammatory potential was measured in terms of the AUC of the graph plotted between the difference in paw diameter and the time. Compared with the % AUC for the untreated paw, which was taken as 100%, oral administration showed swelling of 64.04% while TDS formulation demonstrated 74.16% swelling. Maximum percentage inhibition was observed at

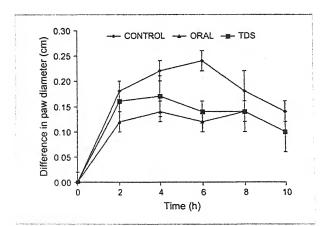


Figure 6: Anti-inflammatory activity of ketorolac on paw edema induced with carrageenan injection (0.05 ml of 0.5% w/w) in rats (control) and after oral administration of ketorolac solution (2 mg/kg) (oral) and on application of the transdermal patch of ketorolac (TDS). The reservoir transdermal patch contained ketorolac (2% w/w), methocel $K_{\tau_5}M$ (2% w/w), isopropyl alcohol (25% w/w), and eucalyptus oil (10% w/w) formulated using phosphate-buffered saline solution of pH 5.4

6h for oral and transdermal application of 50 and 41.67%. The probable reason may be that transdermal delivery is primarily through the paracellular pathway, i.e. around the cells, than through the elastin glue. The glue-like compound, elastin is composed of collagen and hyaluronic acid and other lipids, which occupies the interstices between the cells of the top-most layer of the skin (i.e. the epidermis, including, e.g. stratum corneum, lucidum, granulosum, spinosus) must be dissolved and/or disrupted in order for the drug, dissolved in a solvent, to be able to transverse through the viable skin to the subcutaneous tissues where the cutaneous plexi of the capillary net can be reached. This is achieved only when the permeation enhancer is able to disrupt the upper stratum corneum layer of the skin thus enhancing the permeation rate. The reservoirtype transdermal patch consisting of ketorolac gel thus appears promising in delivering the drug across the skin.

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AQ1: Please give manufacturer information for StatSoft Inc.: town and state.

AQ2: Please define TDS.